Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 820–826
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

An ellagitannin, *n*-butyl gallate, two aryltetralin lignans, and an unprecedented diterpene ester from *Pelargonium reniforme*

Klaus Peter Latté [a], Maki Kaloga [a], Andreas Schäfer [b], Herbert Kolodziej [a,*]

[a] *Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany*
[b] *Freie Universität Berlin, Institute of Organic Chemistry, Takustr. 3, D-14195 Berlin, Germany*

Received 3 July 2007; received in revised form 29 August 2007
Available online 26 October 2007

Abstract

The structural diversity of the metabolic pool of *Pelargonium reniforme* was extended by the characterization of the $^1C_4$-glucose based ellagitannin pelargoniin E, gallic acid *n*-butyl ester, (−)-4,4′,9′-trihydroxy-3′,5′-dimethoxy-2,7′-cyclolignan 9-*O*-β-glucopyranoside and reniformin, a diterpene ester comprised of a diterpene acid with an uncommon –$(CH_2)_2$– bridging element linked to 2-(4-hydroxyphenyl)ethansulfonic acid. These metabolites were associated with the known (α,β)-3,4-di-*O*-galloyl-glucopyranoside, 4,6-dihydroxy-2β-glucopyranosyloxyacetophenone, 1-*O*-galloylglycerol, 6′-*O*-galloylsalidroside and (+)-isolariciresinol-9′-*O*-β-glucopyranoside. All structures were established on the basis of spectroscopic methods.

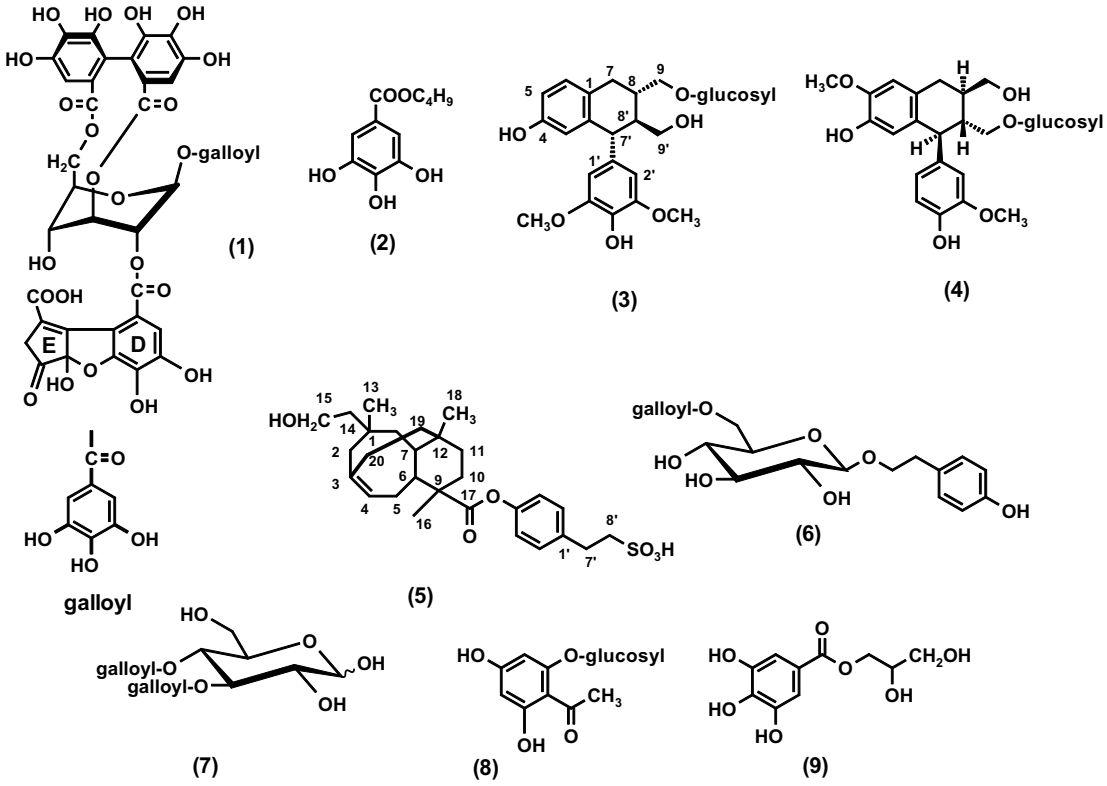
Keywords: *Pelargonium sidoides*; Ellagitannin; Pelargoniin E; Diterpene; Reniformin; Butyl gallate; Aryltetralin lignans

1. Introduction

The pink-flowered *Pelargonium reniforme* CURT. (Geraniaceae), mainly distributed in coastal regions of southern Africa, is an attractive erect shrub and has a long tradition as herbal medicine (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996). Following its therapeutic use in traditional and modern phytomedicine for the treatment of upper respiratory tract infections (Kolodziej and Kiderlen, 2007), this medicinal plant continues to be the subject of extensive studies to identify the underlying active principle(s). The metabolic pool of. *P. reniforme* exhibited remarkable diversity and complexity. Our recent systematic examination has revealed the presence of a notable wealth of highly oxygenated simple coumarins (Latté et al., 2000), ellagitannins with a $^1C_4$ glucose core (Latté and Kolodziej, 2000) and *O*-galloyl *C*-glycosylflavones (Latté et al., 2002). The coumarins and phenolic constituents are of particular interest, displaying moderate antibacterial and fairly high immunomodulatory properties (Kayser and Kolodziej, 1997; Kayser et al., 2001). In our continuing work on this plant, we have discovered three new phenolic metabolites, the corilagin-based ellagitannin pelargoniin E (**1**), *n*-butyl gallate (**2**), and (−)-4,4′,9-trihydroxy-3′,5′-dimethoxy. 2,7′-cyclolignan 9-*O*-β-glucopyranoside (**3**) from the aerial parts, and a novel diterpene ester, reniformin (**5**), from the roots of *P. reniforme*. The former metabolites were associated with five rarely reported compounds (**4**, **6**–**9**). The current paper reports the isolation and structure elucidation of the new natural products.

* Corresponding author. Tel.: +49 30 838 5373; fax: +49 30 838 53729.
E-mail address: kolpharm@zedat.fu-berlin.de (H. Kolodziej).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.08.032



2. Results and discussion

An aqueous acetone extract of the aerial parts of *P. reniforme* was successively extracted with petroleum ether, chloroform and ethyl acetate. The ethyl acetate soluble portion was subjected to repeated chromatography on Sephadex LH-20 using water-methanol gradients. Final purification by semi-preparative HPLC yielded three new natural products, the ellagitannin pelargoniin E (**1**), *n*-butyl gallate (**2**), and the aryltetralin lignan **3** along with five known compounds (**4**, **6**–**9**) obtained for the first time from this plant source.

Compound **1** was readily shown to be an ellagitannin by the characteristic coloration with $FeCl_3$ (blue), potassium iodate (pink) (Haslam, 1965) and sodium nitrite/acetic acid (blue) (Bate-Smith, 1972). Its FAB-MS showed an $[M+Na]^+$ peak at *m/z* 947, reminiscent of pelargoniin A and phyllantusiin C, representing oxidatively modified metabolites of geraniin (Latté and Kolodziej, 2000; Yoshida et al., 1992). Confirmation of the presence of a corilagin moiety was obtained from the fragment at *m/z* 633 and the familiar ^1H resonances of a galloyl group (δ 7.06 *s*, 2xH), a hexahydroxydiphenoyl (HHDP) moiety (δ 6.67 and 6.83, each *s*, 2x1H), and signals characteristic of a $^1C_4$ glucopyranose core (δ 6.44–4.26, 7xH; $J \leqslant 4$ Hz for H-1, H-2, H-3 and H-4). The location of the galloyl group at C-1 followed from a significant downfield shift of the anomeric proton (δ 6.44), assigned with the aid of ^1H–^1H shift correlation spectra, while the *R*-configuration at the chiral HHDP group was evidenced by the negative Cotton effect at 242 nm in the CD spectrum of **1** (Okuda et al., 1995). Besides these signals attributable to a corilagin unit, the ^1H NMR spectrum of **1** showed an isolated methylene function [δ 3.04, br *s*, H_2-3′ (ring E)] and an isolated aromatic proton [δ 7.28, *s* (ring D)], suggesting the presence of an oxidatively modified DHHDP moiety. Given the molecular mass of **1** at *m/z* 924 and the established corilagin moiety in the molecule, the remaining substructure was evidently represented by a $C_{13}H_7O_8$ fragment, compatible with the ion at *m/z* 291 in the EI-MS. Although lack of sufficient sample quantity excluded ^{13}C NMR analysis, comparison of chemical shift data (CD_3OD) with those of the structurally related pelargoniins and realization of a carboxylic proton from an acetone-d_6 spectrum as previously demonstrated for pelargoniin D (Latté and Kolodziej, 2000) in conjunction with close examination of the mass fragmentation of **1** facilitated definition and placement of the modified DHHDP residue. The position of this acyl residue at C-2 was readily inferred from the conspicuous downfield position of H-2 of the glucose moiety (δ 5.23, *d*, $J = 4.0$ Hz) relative to that of corilagin (δ 3.98), while the relatively upfield position of just H-4 Glc (δ 4.45) reflected the only non-acylated position of the carbohydrate residue. The ion at *m/z* 308 indicated the 2-acyloxy fragment resulting from cleavage between the corilagin and the DHHDP moieties. Based on the above evidence, the structure of compound **1** was identified as depicted in its formula. In analogy to the series of structurally related ellagitannins from the same plant source, trivially named pelargoniins A–D (Latté and Kolodziej, 2000), this new analogue was designated as pelargoniin E.

Compound **2**, obtained as a white amorphous powder and visualized by a dark blue colour on silica plates upon treatment with the $FeCl_3$ reagent, was established to have a molecular formula of $C_{11}H_{14}O_5$ by HR-EIMS ($M^{+\cdot}$ at *m/z* 226.2308; calc. 226.2312). Diagnostic features in the ^1H NMR spectrum of **2** were the presence of a two-proton singlet at δ 7.04 for the magnetically equivalent 2- and 6-protons of a galloyl moiety, and sequentially coupled aliphatic proton signals attributable to a methyl group (δ 0.99, *t*, $J = 7.4$ Hz) and three methylene functions (δ 1.48 and 1.72, each m; δ 4.23, *t*, $J = 6.5$ Hz), reminiscent of an *n*-butyl residue. The conspicuous downfield position of one of the aliphatic methylene signals was consistent with the adjacent oxygen of an ester group. Supporting evidence for the presence of these constructing units in **2** was available from the mass fragmentation pattern, showing peaks at *m/z* 170 and 56 for the galloyl and *n*-butyl residue, respectively. Although gallic acid esters are known to widely occur in the plant kingdom, the characterization of **2** is reported from a plant source for the first time.

Compound **3** was found to be a regio- and stereoisomer of the known and concurrently isolated (+)-isolariciresinol 9′-*O*-β-glucopyranoside (**4**) as indicated by identical $[M+H]^+$ peaks at *m/z* 523 in the FAB-MS, the optical rotations {$[\alpha]^{20}$ −56.0 ($c = 0.06$ in MeOH) and +47.6 ($c = 0.04$ in MeOH) for **3** and **4**, respectively} and the analogy of signal and coupling patterns of their ^1H NMR spectra. Notable differences included replacement of the feruloyl substitution pattern on the pendent 7′-aryl entity by a syringoyl moiety as concluded from a two-proton aromatic singlet (δ 6.77) and two methoxyl resonances (δ 3.83 and 3.86), reminiscent of that of lyoniresinol and 5′-methoxy-isolariciresinol derivatives (Dictionary of Natural Products on CD-ROM, 2006). The ABX-system in the aromatic region verified the 1,3,4-substitution pattern of the remaining aryl ring of the lignan framework. Notably, this arrangement has been encountered for the first time for a member of aryltetralin lignans. Coupling constants for the aliphatic protons of **3** ($J_{7',8'} = J_{8',8} = 7.1$ Hz) reflected *trans–trans* configuration, while the negative sign of the optical rotation strongly supported a structure analogous to *ent*-isolariciresinol (Urones et al., 1987; Vecchietti et al., 1979) and (−)-lyoniresinol derivatives (Lundgren et al., 1981 and 1985; Ohashi et al., 1994; Smite et al., 1995; Cortez et al., 1998). Based on the dihedral angles derived from Dreiding models and application of the Karplus equation, the conspicuously small *J* values were attributable to a half-chair conformation of the cyclohexene ring with the pendent 1-aryl ring in an axial orientation. Note, however, that although a relative *trans–trans* stereochemistry of **4** was evident from large coupling constants ($J_{7',8'} = J_{8',8} = 10.8$ Hz), the 7′-aryl group adopted an equatorial position. The ^1H NMR spectrum of **3** also showed seven aliphatic proton signals of a carbohydrate unit, with coupling constants consistent with a β-D-glucopyranose, upon the assumption that it is of the D-form. Owing to insufficient sample quantity for ^{13}C NMR analysis and extended 2D NMR experiments, the assignment of the position of glycosylation at C-9 was concluded from comparing the chemical shifts of H_2-9′ (δ 3.23 and 4.05) and H_2-9 (δ 3.71 and 3.76) of **3** with those of **4** (δ 3.54, 3.93 and at ca. 3.50, respectively) with established structure. Thus, the conspicuous upfield shifts of the H_2-9′ signals ($\Delta\delta$ ca 0.25 ppm) suggested that the glucosyl moiety could be placed on the hydroxyl at C-9. Compound **3** was, therefore, identified as the new naturally occurring aryltetralin lignan, (−)-4,4′,9′-trihydroxy-3′,5′-dimethoxy-2,7′-cyclolignan 9-*O*-β-glucoside.

An aqueous acetone extract of the roots of *P. reniforme* was similarly partitioned between water and organic solvents of increasing polarity as noted above. The extractives of the ethyl acetate phase were repeatedly subjected to chromatography on Sephadex LH-20 using water–methanol gradient systems. Subsequent purification of appropriate fractions, combined according to TLC monitoring, by HPLC on RP-18 material with water containing increasing amounts of methanol afforded the novel diterpene ester (**5**), designated as reniformin.

Compound **5** was isolated as a white amorphous solid, possessing the empirical formula $C_{28}H_{40}SO_6$ as concluded from an $[M-H]^-$ ion peak at *m/z* 503 in the negative FAB-MS and the key diterpene fragment $[M-(C_8H_{10}SO_4)]^{+\cdot}$ observed at *m/z* 302.22444 (calc. 302.22458 for $C_{20}H_{30}O_2$) in the HREIMS (Fig. 1). Analysis of its ^1H NMR spectrum revealed the presence of an AA′BB′-spin system at δ 6.76 and 7.09 (H-3′/H-5′ and H-2′/H-6′, respectively; each *d*, $J = 8.4$), typical of a *p*-disubstituted benzene entity and consistent with the presence of a 2-(4-hydroxyphenyl)ethansulfonic moiety. This partial structure was further evidenced by the long-range correlations between C-1′ (δ_C 128.3) and two mutually coupled methylene groups, assigned to C-7′ (δ 2.84) and C-8′ (δ 3.09). The conspicuous downfield position of the latter signal agreed well with the vicinity of the SO_3H functionality. Consistent with this placement of the sulfonate group was also the deshielded C-8′ resonance (δ_C 42.2) relative to that of C-7′ (δ_C 33.8) (Yao, 2003; Yin et al., 2005). Taking into account the elemental composition of **5** (Section 3), the HR-EIMS data of the diterpenoid moiety (Fig. 1) and the nature of the ester bond established through HMBC experiments (vide infra), the placement of the sulfonate group at C-8′ was beyond doubt. On the basis of these data, the structure of the C-9 substituent was elucidated as 2-(4-hydroxyphenyl)ethansulfonic entity. It should be noted that this residue has not been reported to date for natural products.

Closer analysis of the ^1H and ^{13}C NMR spectra suggested the presence of a rearranged diterpene skeleton. Unambiguous proton and carbon signal assignments were facilitated by extensive 2D NMR experiments (COSY, HETCOR, HMBC, HMQC), though partially severe overlapping of proton signals in the ^1H NMR spectrum was apparent. From the ^{13}C and DEPT NMR spectra, 28 resonances were clearly discernable, for $3 \times CH_3$, $11 \times CH_2$, $7 \times CH$ and $7 \times C$. A diagnostic feature in the ^{13}C NMR

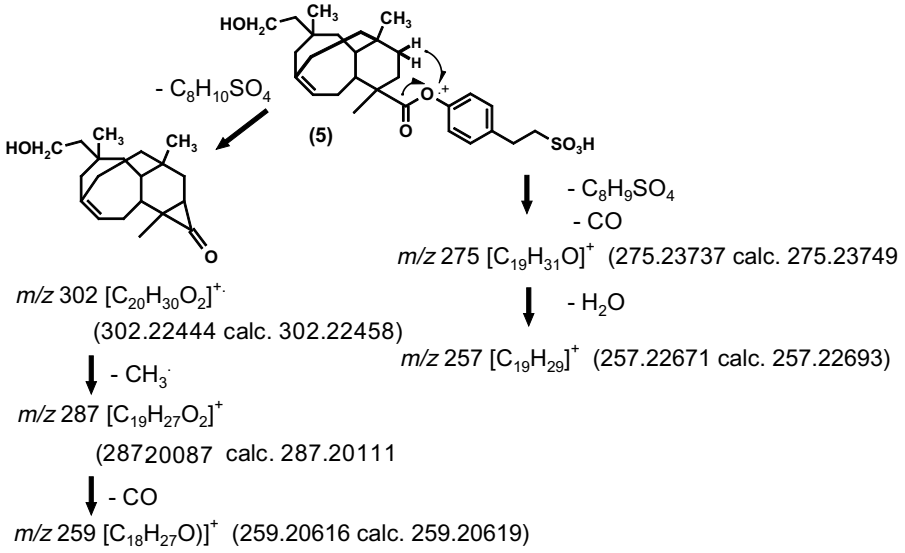


Fig. 1. Proposed mass spectral fragmentation of **5** and HR-EIMS data of key fragments.

spectrum was the appearance of an ester carbonyl signal (δ_C 181.5). HMBC experiments correlated the low-field ester carbonyl carbon with H_a-10 (δ 1.04), H-6 (δ 1.34) and the methyl protons at C-16 (δ 1.19). These three-bond associations thus provided strong evidence for the placement of the ester carbonyl at C-9 of the diterpene moiety and the mode of bonding between this entity and 2-(4-hydroxyphenyl)ethansulfonic acid.

Further detailed analysis of the observed HETCOR and HMQC/HMBC correlations facilitated definition of the novel diterpene skeleton (Table 1). The well separated proton signal of the olefinic methine H-4 (δ 5.35) and the *tert*-

Table 1
^{1}H and ^{13}C NMR data of **5** and the associated HMBC correlations[a]

No.	^{1}H (δ, J in Hz)	^{13}C (δ_C)	HMBC correlations
1		34.05	H_3-13, H_2-14, H_2-15
2	1.91, 2H, br *s*	48.35	H_3-13, H_2-14
3		136.12	H_2-2, H_2-20
4	5.35, 1H, *m*	122.42	H_2-2, H_2-20
5	2.13, 1H, br *d* (13.5) 2.42, 1H, *t*-like (14.0)	25.53	H-6
6	1.34, 1H, *dd* (12.3 and 4.1)	52.80	H_3-16, H-4
7	1.67, 1H, *m*	52.90	H-6, H_3-18
8	1.29, 1H, *m* 1.55, 1H, *m*	38.22	H_3-13, H_2-14
9		44.66	H-6, H_3-16
10	1.04, 1H, *dd* (13.5 and 3.4) 2.13, 1H, br *d* (13.5)	39.40	H_2-11, H_3-16
11	1.44, 1H, *m* 1.96, 1H, *m*	20.83	H_3-18
12		36.89	H-6, H_3-18
13	0.81, 3H, *s*	22.08	H_2-14
14	1.58, 2H, *t*-like (7.4)	44.80	H_3-13, H_2-15
15	4.09, 2H, *t*-like (7.4)	65.91	H_2-14
16	1.19, 3H, *s*	29.77	H-6, H_a-10
17		181.53	H-6, H_a-10, H_3-16
18	0.77, 3H, *s*	14.78	
19	1.09 1H, br *dd* (13.2 and 4.0) 1.88, br *d* (13.2)	41.10	H_2-11, H_3-18
20	1.27, 1H, *m*[b] 1.54, 1H, *m*	22.08	
1′		128.33	H-3′, H-5′, H_2-7′, H_2-8′
2′	7.09, 1H, *d* (8.5)	130.81	H_2-7′
3′	6.76, 1H, *d* (8.5)	116.70	
4′		157.80	H-2′, H-3′, H-5′, H-6′
5′	6.76, 1H, *d* (8.5)	116.70	
6′	7.09, 1H, *d* (8.5)	130.81	H_2-7′
7′	2.84, 2H, *t*-like (7.5)	33.80	H_2-8′
8′	3.09, 2H, *t*-like (7.5)	42.24	H_2-7′

[a] Data were obtained in CD_3OD at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR.
[b] Overlapping with H_a-8.

methyl singlet (H_3-16, δ 1.19) in the 2D HMBC plot were observed to be associated with C-6 (δ_C 52.8). In addition, the H-6 signal (δ 1.34) showed distinct two-bond correlations to C-5 (δ_C 25.5), C-7 (δ_C 52.9) and C-9 (δ_C 44.7), while that of H_3-16 was associated with the quaternary C-9. Correlations from H_2-2 (δ 1.91) to both C-3 (δ_C 136.1) and C-4 (δ_C 122.4) established the proposed arrangement.

When taken in conjunction with the observed couplings between H_2-19 and H_2-20 that was evident from a COSY spectrum, the cross peak between C-19 (δ_C 41.1) and H_2-11 (δ 1.44 and 1.96) and a distinct long-range correlation from C-19 to H_3-18 in the HMBC experiment established the unusual C-3/C-12 bridging in **5**. Such a remarkable mode of linkage was additionally confirmed by the crucial long-range correlations between H_2-20 and both C-3 and C-4. The presence of an oxygen bearing ethyl group was inferred from the remaining ^{13}C resonances at δ_C 44.8 (C-14) and 65.9 (C-15) and from the chemical shifts in the ^1H NMR spectrum of H_2-14 (δ 1.58) and H_2-15 (δ 4.09). Location of this side chain at C-1 followed from long-range correlations between C-2 (δ_C 48.4) and the H_2-14 and H_3-13 signals. Consistent with the above placement, HMBC experiments also showed definite associations between C-13 (δ_C 22.1) and H_2-14, and between C-1 (δ_C 34.05) and H_2-15 (δ 4.09).

Collectively these spectral data and other significant HMBC correlations (Table 1) defined the novel diterpene ester with an uncommon –CH_2–CH_2-bridging element as depicted in structure **5**. The isolation and structure elucidation of **5** not only extends the range of known diterpene skeletons but also introduces a new substituent, 2-(4-hydroxyphenyl)ethansulfonic acid, that has not yet been reported for natural products. For simplicity, this unique diterpenoid **5** was named reniformin. Note that compound **5** was apparently accompanied by analogous metabolites. Low quantities and the gradual decomposition excluded the isolation and structural assessment of additional fascinating analogues.

The known compounds **4** and **6**–**9** were identified by comparison of their physical data with those reported in the literature. Since some of their spectral data were not previously recorded, these are included for comparative purposes (see Section 3). Noteworthy is that these compounds represent rarely reported metabolites. The presence of the lignan **4** has only been demonstrated in *Populus nigra* (Thieme and Benecke, 1969) and *Stemmadenia minima* (Achenbach et al., 1992). Compound **6** has previously been reported merely from two *Quercus* species (Nonaka et al., 1982,1989), **7** from *Macaranga tamarius* (Lin et al., 1990), **8** from *Curcuma comosa* (Suksamrarn et al., 1997) and *Myrcia multiflora* (Yoshikawa et al., 1998), and **9** from some *Rheum* species (Nonaka and Nishioka, 1983) and *Mallotus japonicus* (Saijo et al., 1990).

The results presented in this and preceding papers (Latté and Kolodziej, 2000; Latté et al., 2000, 2002) demonstrate a remarkable structural diversity amongst constituents of *P. reniforme* and indicate that *Pelargoniums* are a rich source of attractive natural products.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin–Elmer 2M/MC polarimeter, and CD spectra on a Jasco J-720W spectrometer. Elemental analysis was acquired with a Vario EL facility. EI- and FABMS were obtained on a Varian MAT CH_2A and a Finnigan MAT CH_5DF mass spectrometer, respectively, while HR-EIMS data were measured on a Finnigan MAT 711. ^1H (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on a Bruker AC-400 instrument, and chemical shifts are given in δ (ppm) values relative to that of $(Me)_4Si$. HMBC experiments were recorded on a Bruker AMX-400 spectrometer, optimized for $^{2-3}J_{H/C} = 8$ Hz. HPLC separations were conducted on a Knauer instrument, equipped with a gradient former and a variable UV detector, and computer integrating model (EuroChrom 2000). Experimental conditions: Eurospher 100C-18 (5 μm; 8 × 250 mm); mobile phase, H_2O–MeOH gradient 9:1 → 3:7 (40 min, flow rate 4 ml/min, detection at 275 nm). Column chromatography was performed with Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd.), using a H_2O–MeOH gradient system (1:0 → 0:1), and TLC analysis was achieved with Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) with EtOAc–HCOOH–H_2O (18:1:1) as developing system.

3.2. Plant material

The plant material of *P. reniforme* was kindly provided by Dr. Willmar Schwabe GmbH & Co, Karlsruhe, Germany. A voucher specimen has been deposited at the Institute of Pharmacy, Pharmaceutical Biology, Freie Universität Berlin.

3.3. Extraction and isolation

The dried aerial parts of *P. reniforme* (4.1 kg) were exhaustively extracted with acetone–H_2O (4:1 V/V). The combined extracts were reduced in volume and defatted with petroleum ether (16 × 500 ml). The aqueous phase was subsequently successively treated with $CHCl_3$ and EtOAc, and the EtOAc extractives (32.3 g) subjected to chromatography on Sephadex LH-20 and HPLC separations as described (Latté and Kolodziej, 2000) to afford compounds **1**–**4** and **6**–**9** in amounts of 1–3 mg. The purification described for the individual compounds follows initial chromatography on Sephadex LH-20 with a gradient solvent system of H_2O–MeOH (9:1 → 0:1).

The aqueous acetone extract of the dried and powdered roots (1.5 kg) was similarly partitioned with organic solvents of increasing polarity. The EtOAc soluble portion

(10 g) was chromatographed on Sephadex LH-20 (120 × 4 cm) by first eluting with water then followed with increasing amounts of MeOH. The fraction 316–430 ml eluted with H_2O–MeOH (9:1) was re-chromatographed on Sephadex LH-20 (120 × 2.5). From eluants 66–130 ml compound **5** (12 mg) was obtained, following purification by HPLC on RP-18 (H_2O–MeOH, 9:1 → 3:7).

3.4. Pelargoniin E (*1*)

The content (10 ml fractions) of test tubes 491–590 (70 mg) was subjected to HPLC separation to afford **2**. A white amorphous powder, R_f 0.42, R_t 19.2 min; $[\alpha]_D^{20}$ −96.7 ($c = 0.18$ in MeOH). FAB-MS: *m/z* 947 $[M+Na]^+$, 291. EIMS: *m/z* (rel. int. %) 308 (8). ^1H NMR (CD_3OD): δ 7.28 (*s*, H-3 ring D), 7.06 (2H, *s*, galloyl H-2/H-6), 6.83 (*s*, HHDP, H-3), 6.67 (*s*, HHDP, H-3́), 6.44 (*d*, $J = 4.0$, Glc H-1), 5.23 (br *d*, $J = 4.0$, Glc H-2),4.85 (Glc H-3, overlapped with solvent), 4.64 (*t*-like, $J = 10.5$, Glc H_b-6), 4.55 (*t*-like, $J = 7.5$, Glc H-5), 4.45 (br *d*, $J = 2.9$, Glc H-4), 4.26 (*dd*, $J = 7.5$ and 10.5, Glc H-6_a), 3.04 (2H, *s*, H_2-3 ring E). CD $[\Theta]_{242}$ −47,000, $[\Theta]_{271}$ +30,000, $[\Theta]_{295}$ −18,000.

3.5. n-Butyl gallate (*2*)

The content (10 ml fractions) of test tubes 2100–2300 (268 mg) was re-chromatographed on Sephadex LH-20 using a H_2O–MeOH gradient system (8:2 → 5:5). HPLC separation of the eluant 160–212 ml (43 mg) afforded **3**. A white amorphous powder, R_f 0.91, R_t 24.6 min; m.p. 150 °C. (−)-FAB-MS: *m/z*: 225 $[M-H]^-$. ^1H NMR (CD_3OD): δ 7.04 (2H, *s*, H-2/H-6), 4.23 (2H, *t*, $J = 6.51$, H_2-1′), 1.72 (2H, *m*, H_2-2′), 1.48 (2H, *m*, H_2-3′), 0.99 (3H, *t*, $J = 7.4$, H_3-4′).

3.6. (−)-4,4′,9′-Trihydroxy-3′,5′-dimethoxy-2,7′-cyclolignan 9-O-β-glucopyranoside (*3*)

A white amorphous powder, R_f 0.24, R_t 10.6 min. $[\alpha]_D^{20}$ −56.0 ($c = 0.06$, MeOH). (+)-FAB-MS: *m/z* 545 $[M+Na]^+$, 523 $[M+H]^+$. ^1H NMR (CD_3OD, 600 MHz): δ 6.97 (*d*, $J = 1.9$, H-3), 6.85 (*dd*, $J = 8.6$, 1.9, H-5), 6.79 (*d*, $J = 8.6$, H-6), 6.77 (2H, *s*, H-2′/H6′), 4.27 (*d*, $J = 7.8$, Glc H-1), 3.93 (*dd*, $J = 5.5$, 6.4, H_b-9′), 3.88 (*s*, 3′-OCH_3), 3.87 (*m*, Glc H_b-6), 3.83 (*s*, 6-OCH_3), 3.79 (br *d*, $J = 7.1$, H-7′), 3.67 (*dd*, $J = 11.1$, 5.2, Glc H_a-6), 3.54 (*dd*, $J = 10.0$, 5.5, H_a-9′), 3.51-3.46 (3H, *m*, H_a-9, H_b-9, H_b-7), 3.40-3.24 (3H, *m*, Glc H-3, Glc H-4, Glc H-5), 2.70 (*t*-like, $J = 7.1$, H-8), 1.93 (2H, *m*, H-8′, H_a-7).

3.7. (+)-Isolariciresinol-9′-β-glucopyranoside (*4*)

A white amorphous powder, R_f 0.20, R_t 14.3 min. $[\alpha]_D^{20}$ +47.6 ($c = 0.04$ in MeOH). (+)-FAB-MS: *m/z* 523 $[M+H]^+$; HREIMS *m/z* 522.2104, calc. for $C_{26}H_{34}O_{11}$ 522.2101. ^1H NMR (CD_3OD, 600 MHz): δ 6.77 (*d*, $J = 1.9$, H-2′), 6.73 (*d*, $J = 8.1$, H-5′), 6.64 (br *s*, H-6), 6.61 (*dd*, $J = 8.1$, 1.9, H-6′), 6.16 (br *s*, H-3), 4.11 (*d*, $J = 7.8$, Glc H-1), 4.07 (br *d*, $J = 10.8$, H-7′), 4.05 (*dd*, $J = 10.2$, 2.4, H_b-9′), 3.82 (*dd*, $J = 12.0$, 2.4, Glc H_b-6), 3.80 (*s*, 6-OCH_3), 3.79 (*s*, 3′-OCH_3), 3.76 (*dd*, $J = 11.2$, 4.0, H_b-9), 3.71 (*dd*, $J = 11.2$, 6.3, H_a-9), 3.64 (*dd*, $J = 12.0$, 5.4, Glc H_a-6), 3.35 (*t*-like, $J = 9.0$, Glc H-3), 3.26 (*t*-like, $J = 9.0$, Glc H-4), 3.23 (*dd*, $J = 10.2$, 3.6, H_a-9′), 3.20-3.17 (2H, *m*, Glc H-2/H-5), 2.84 (*dd*, $J = 16.0$, 10.4, H_b-7), 2.79 (*dd*, $J = 16.0$, 6.0, H_a-7), 2.08 (*m*, H-8), 1.85 (*tt*-like, $J = 10.8$, 3.6, 2.4, H-8′); ^{13}C NMR (CD_3OD, 150 MHz): δ 148.9 (C-3′), 147.2 (C-5), 145.8 (C-4́), 145.2 (C-74), 138.7 (C-1′), 134.4 (C-2), 129.1 (C-1), 123.1 (C-6′), 117.4 (C-3), 116.1 (C-5′), 114.3 (C-2′), 112.4 (C-6), 105.2 (Glc C-1), 78.1 (Glc C-3), 77.9 (Glc C-5), 75.2 (Glc C-2), 71.7 (Glc C-4), 69.5 (C-9′), 65.2 (C-9), 62.8 (Glc C-6), 56.5 (6-OCH_3), 56.4 (3′-OCH_3), 47.9 (C-7′), 45.9 (C-8′), 39.5 (C-8), 33.9 (C-7).

3.8. Reniformin (*5*)

A white amorphous powder (12 mg); R_t 29.6 min; [α]D +33 (c 0.03, MeOH).(Found C, 66.6; H, 7.9; S, 6.1. $C_{28}H_{40}SO_6$ requires C, 66.6; H, 8,0; S, 6.3). (−)-FABMS *m/z* 503 $[M-H]^-$; HR-EIMS *m/z* 302.22444 [M^+-2-(4-hydroxyphenyl)ethansulfonic acid, calc. for $C_{20}H_{30}O_2$ 302.22458]. For further fragments see Fig. 1. ^1H and ^{13}C NMR data see Table 1.

3.9. (α,β)-3,4-Di-O-galloylglucopyranoside (*6*)

A white amorphous powder, R_f 0.30, R_t 11.4 min. (−) FAB-MS: *m/z*: 483 $[M-H]^-$. ^1H NMR (CD_3OD): δ 7.14 and 7.09 (2 × *s*, 2 × Galloyl H-2/H-6); β-isomer: 5.42 (*t*, $J = 9.6$, Glc H-3), 5.14 (*t*, $J = 9.0$, Glc H-4), 4.87 (*d*, $J = 8.8$, Glc H-1), 4.5–3.5 (3H, *m*, Glc H-2/H2-6), 4.19 (*m*, Glc H-5); α-isomer: 5.74 (*t*, $J = 10.0$, Glc H-3), 5.34 (*d*, $J = 4.0$, Glc H-1), 5.17 (*m*, Glc H-4), 4.5-3.5 (4H, *m*, Glc H-2, Glc H-5, Glc H_2-6).

3.10. 4,6-Dihydroxy-2-β-glucopyranosyloxyacetophenone (*7*)

A white amorphous solid, R_f 0.39, R_t 7.3 min. (−)-FAB-MS: *m/z*: 329. $[M-H]^-$. ^1H NMR (CD_3OD): δ 6.18 (*d*, $J = 2.2$, H-5), 5.94 (*d*, $J = 2.2$, H-3), 5.04 (*d*, $J = 7.6$, Glc H-1), 3.91 (*dd*, $J = 12.0$, 1.8, Glc H_b-6), 3.72 (*dd*, $J = 5.0$, 12.0, Glc H_a-6), 3.50–3.30 (4H, *m*, Glc H-2–H-5), 2.70 (*s*, 8-OCH_3). Physical data corresponded to those in the literature (Yoshikawa et al., 1998).

3.11. 1-O-Galloylglycerol (*8*)

A white amorphous powder, R_f: 0.55; R_t: 3.3 min. Mp. 185-187 °C, $[\alpha]^{20}$ +2.8 ($c = 0.5$ in MeOH). (−)-FAB-MS: *m/z*: 243 $[M-H]^-$. ^1H NMR (CD_3OD): δ 7.08 (2H, *s*, H-2/H-6), 4.29 (2H, *m*, H_2-1′), 3.92 (2H, *m*, H-2′), 3.62 (2H,

m, H_2-3′). Physical data corresponded to those in the literature (Nonaka and Nishioka, 1983).

3.12. 6′-*O*-Galloylsalidroside (**9**)

An off-white solid, R_f 0.39; R_t 12.1 min. m.p. 115–116 °C. [α_D^{20} −16.6 ($c = 0.11$ in MeOH). (−)-FAB-MS: *m*/*z*: 451 [M−H]$^-$, 331 [(M−H)-4-vinylphenol]$^-$, 298 [(M−H)-galloyl]$^-$. ^{1}H NMR (CD_3OD): δ 7.09 (2H, *s*, galloyl H-2/H-6), 6.98 (2H, *d*, $J = 8.5$, H-3/H-5), 6.65 (2H, *d*, $J = 8.5$, H-2/H-6), 4.58 (*dd*, $J = 2.0$, 11.9, Glc H_b-6), 4.43 (*dd*, $J = 5.7$, 11.9, Glc H_a-6), 4.32 (*d*, $J = 7.8$, Glc H-1), 3.92 (*m*, H_b-1), 3.70 (*m*, H_a-1), 3.55 (*m*, Glc H-5), 3.39 (2H, *t*, $J = 8.0$, Glc H-3, Glc H-4), 3.21 (*t*, $J = 8.4$, Glc H-2), 2.81 (2H, *m*, H_2-2). ^{13}C NMR (CD_3OD): δ_C 168.4 (COOH), 156.7 (C-4), 146.6 (galloyl C-3/C-5), 139.1 (galloyl C-4), 130.9 (C-2/C-6), 130.7 (C-1), 121.5 (galloyl C-1), 116.1 (C-3/C-5), 110.2 (galloyl C-2/C-6), 104.6 (Glc C-1), 78.0 (Glc C-3), 75.5 (Glc C-5), 75.1 (Glc C-2), 72.3 (Glc C-4), 71.8 (C-2), 64.8 (Glc C-6), 36.5 (C-1).

Acknowledgements

The authors are grateful to Dr. K. Bergander, Institut für Organische Chemie, Westfälische Wilhelms-Universität Münster, Germany, for recording 600 MHz NMR spectra on a Varian unity Inova spectrometer and performing HMBC experiments. Thanks are also due to Prof. T. Yoshida, Okayama University, Japan, for recording the CD spectrum of pelargoniin E.

References

Achenbach, H., Löwel, M., Waibel, R., Gupta, M., Solis, P., 1992. New lignan glucosides from *Stemmadenia minima*. Planta Med. 58, 270–272.

Bate-Smith, E.C., 1972. Detection and determination of ellagitannins. Phytochemistry 11, 1153–1156.

Cortez, D.A.G., Fernandes, J.B., Cass, Q.B., Vieira, P.C., Da Silva, M., Fatima, G.F., Ferreira, A.G., Pirani, J.R., 1998. Lignan glycosides from *Trichilia estipulata* bark. Nat. Prod. Lett. 11, 255–262.

Dictionary of Natural Products on CD-ROM, 2006. Version 15:2. Chapman and Hall, CRC Press, London, UK.

Haslam, E., 1965. Galloyl esters in the aceraceae. Phytochemistry 4, 495–498.

Hutchings, A., 1996. Zulu Medicinal Plants. Natal University Press, Pietermaritzburg.

Kayser, O., Kolodziej, H., 1997. Antibacterial activity of extracts and constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Planta Med. 63, 508–510.

Kayser, O., Kiderlen, A.F., Kolodziej, H., 2001. Immunomodulatory principles of *Pelargonium sidoides*. Phytother. Res. 15, 122–126.

Kolodziej, H., Kiderlen, A.F., 2007. *In vitro* evaluation of antibacterial and immunomodulatory activities of *Pelargonium reniforme*, *Pelargonium sidoides* and the related herbal drug preparation EPs® 7630. Phytomedicine 14 (Suppl. VI), 18–26.

Latté, K.P., Kolodziej, H., 2000. Pelargoniins, new ellagitannins from *Pelargonium reniforme*. Phytochemistry 54, 701–708.

Latté, K.P., Kayser, O., Tan, N., Kaloga, M., Kolodziej, H., 2000. Unusual coumarin patterns of *Pelargonium* species forming the origin of the traditional herbal medicine umckaloabo. Z. Naturforsch. 55c, 528–533.

Latté, K.P., Ferreira, D., Venkatraman, M.S., Kolodziej, H., 2002. *O*-Galloyl-*C*-glycosylflavones from *Pelargonium reniforme*. Phytochemistry 59, 419–424.

Lin, J.H., Nonaka, G., Nishioka, I., 1990. Tannins and related compounds. XCIV. Isolation and characterization of seven new hydrolysable tannins from the leaves of *Macaranga tanarius* (L.). MUELL. et ARG. Chem. Pharm. Bull. 38, 1218–1223.

Lundgren, L.N., Popoff, T., Theander, O., 1981. Dilignol glycosides from needles of *Picea abies*. Phytochemistry 20, 1967–1969.

Lundgren, L.N., Shen, Z., Theander, O., 1985. The constituents of conifer needles: Part 12. Dilignol glycosides from *Pinus massoniana* lamb. Acta Chem. Scand. B 39, 241–248.

Nonaka, G., Nishioka, I., 1983. Tannins and related compounds. Part X. Rhubarb (2): Isolation and structures of a glycerol gallate, gallic acid glucosides gallates, galloylglucoses and isolindleyin. Chem. Pharm. Bull. 31, 1652–1658.

Nonaka, G., Nishimura, H., Nishioka, I., 1982. Tannins and related compounds. Part IV. Seven new phenol glucoside gallates from *Quercus stenophylla* MAKINO (1). Chem. Pharm. Bull. 30, 2061–2067.

Nonaka, G., Nakayama, S., Nishioka, I., 1989. Tannins and related compounds. Part LXXXIII. Isolation and structures of hydrolyzable tannins, phillyraeoidins A–E from *Quercus phillyraeoides*. Chem. Pharm. Bull. 37, 2030–2036.

Ohashi, K., Watanabe, H., Okumura, Y., Uji, T., Kitagawa, I., 1994. Indonesian medicinal plants. XII. Four isomeric lignan-glucosides from the bark of *Aegle marmelos* (Rutaceae). Chem. Pharm. Bull. 42, 1924–1926.

Okuda, T., Yoshida, T., Hatano, T., 1995. Hydrolyzable tannins and related polyphenols. Fortschr. Chem. Org. Naturst. 66, 1–125.

Saijo, R., Nonaka, G., Nishioka, I., 1990. Gallic acid esters of bergenin and norbergenin from *Mallotus japonicus*. Phytochemistry 29, 267–270.

Smite, E., Pan, H., Lundgren, L.N., 1995. Lignan glycosides from inner bark of *Betula pendula*. Phytochemistry 40, 341–343.

Suksamrarn, A., Eiamong, S., Piyachaturawat, P., Byrne, L.T., 1997. A phloracetophenone glucoside with choleretic activity from *Curcuma comosa*. Phytochemistry 45, 103–105.

Thieme, H., Benecke, R., 1969. Isolierung und Konstitutionsaufklärung eines Cyclolignanglucosids aus den Blättern von *Populus nigra*. L. Pharmazie 24, 567–572.

Urones, J.G., De Pascual Teresa, J., Sanchos Marcos, I., Diez Martin, D., 1987. Ent-isolariciresinol in *Reseda suffruticosa*. Phytochemistry 5, 1540–1541.

Vecchietti, V., Ferrari, G., Orsini, F., Pelizzoni, F., 1979. Alkaloid and lignan constituents of *Cinnamosma madagascariensis*. Phytochemistry 18, 1847–1849.

Watt, C., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edinburgh, London, 449.

Yao, X., 2003. Identification of a rare sulfonic acid metabolite of andrographolide in rats. Drug Metabol. Disposit. 31, 983–985.

Yin, H., Fu, H., Hua, H., Qi, X., Li, W., Sha, Y., Pei, Y., 2005. Two new sesquiterpene lactones with the sulfonic acid group from *Saussurea lappa*. Chem. Pharm. Bull. 53, 841–842.

Yoshida, T., Ito, H., Matsunaga, S., Tanaka, R., Okuda, T., 1992. Tannins and related polyphenols of Euphorbiaceous plants. IX. Hydrolyzable tannins with[1] C_4 glucose core from *Phyllanthus flexuosus*. Muell Arg. Chem. Pharm. Bull. 40, 53–60.

Yoshikawa, M., Shimada, H., Nishida, N., Li, Y., Toguchida, I., Yamara, J., Matsuda, H., 1998. Antidiabetic principles of natural medicines. II. Aldose reductase and α-glucosidase inhibitors from Brazilian natural medicine, the leaves of *Myrcia multiflora* DC. (Myrtaceae): structures of myrciacitrins I and II and myrciaphenones A and B. Chem. Pharm. Bull. 46, 113–119.