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Pelargoniins, new ellagitannins from *Pelargonium reniforme*

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Abstract

The range of natural ellagitannins is extended by identification of five new metabolites with $^1C_4$ glucose core, designated as pelargoniins A–D and isocorilagin, and the new phyllanthusiin E methyl ester. They are accompanied in the aerial parts of *Pelargonium reniforme* by two known structurally related metabolites, corilagin and phyllanthusiin C, two phenolcarboxylic acids, brevifolincarboxylic acid and phyllanthusiin E, the gallotannin 1-*O*-galloyl-β-D-glucopyranose, and the ellagitannins strictinin and isostrictinin having a $^4C_1$-glucose core. The structures of these compounds were established from spectroscopic studies. This is the first example of the co-occurrence of ellagitannins with $^4C_1$ and $^1C_4$ glucopyranose core demonstrated for a member of the Geraniaceae.

Keywords: *Pelargonium reniforme*; Geraniaceae; Ellagitannins; Pelargoniins; Isocorilagin; Phyllanthusiin E methyl ester

1. Introduction

The importance of the plants in the genus *Pelargonium* in traditional medicine is well documented and these form the basis of herbal medicines in areas of southern Africa (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996). The species *Pelargonium reniforme* CURT. enjoys a wide reputation among the native population for its curative or palliative effects in the treatment of gastrointestinal, hepatic and respiratory tract disorders. The aerial parts of the titled plant are employed in wound healing which may be explained, at least in part, by the presence of tannins in fairly high concentrations. At present there is no report on the structural assessment of individual tannins from *Pelargoniums*, their characterisation being hitherto limited to colourations with chemical reagents (Bate-Smith, 1973). In continuation of our earlier studies on the chemistry of *Pelargonium* species (Kayser and Kolodziej, 1995), and in search for the active principles of claimed indications (Kayser et al., 2000), we report herein on the isolation and structure elucidation of a series of hydrolysable tannins including new geraniin-related ellagitannins from the aerial parts of *P. reniforme*.

2. Results and discussion

The aqueous acetone extract of the aerial parts of *Pelargonium reniforme* was successively extracted with petroleum ether, chloroform, ethyl acetate and *n*-butanol. The extractives of the ethyl acetate and *n*-butanol phases were separately fractionated by a combination of chromatography on Sephadex LH-20 and RP-18 material using gradient systems of water–methanol to afford compounds **1**–**13**, including six new hydrolysable tannins.

Known compounds amongst the polyphenolic metabolites included the phenolcarboxyclic acids brevifolincarboxylic acid (**1**), phyllanthusiin E (**2**), the gallotannin 1-*O*-galloyl-β-D-glucopyranose (**4**), the ellagitannins strictinin (**5**), isostrictinin (**6**), corilagin (**7**), and phyllanthusiin C (**9**), which were readily identified by comparison of the physical properties with

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those reported in the literature. Noteworthy is that compounds **2** and **9** represent rarely found metabolites, their occurrence being hitherto confined to a few euphorbiaceous plants. The presence of **2** has only been demonstrated in *Phyllanthus flexuosus* (Yoshida et al., 1992), while two additional sources of compound **9** were recently reported, viz. *Acalypha hispida* (Amakura et al., 1999) and *P. myrtifolius* (Liu et al., 1999).

Although the structure of corilagin (**7**) is firmly established as 1-*O*-galloyl-3,6-(*R*)-hexa-hydroxydiphenoyl (HHDP) -($^1C_4$/1B)-β-D-glucopyranose (Okuda et al., 1982a; Nawwar et al., 1994), the present detection of its α-isomer (**8**) renders previous characterisations ambiguous that were lacking crucial data for definition of stereochemistry. The ^{1}H NMR spectrum (CD_3OD) of **8** was virtually superposable with that of **7**, while the R-configuration at the chiral HHDP group was evidenced by similar negative Cotton effects at 246 in the CD spectrum in each instance. Definition of the α-orientation was concluded from spectroscopic and chromatographic evidence. Notable differences included the optical rotation $[\alpha]_D^{20}$ −53.6° ($c = 0.11$ in MeOH) and $[\alpha]_D^{20}$ −250.0° ($c = 0.3$ in MeOH) for **8** and **7**, respectively} and chromatographic properties on silica plates (R_f 0.3 versus 0.5) and on RP-18 material (R_t 8.3 versus 9.9 min) (cf. Section 3). With DMSO-d_6 as solvent, the ^{1}H NMR spectrum of an authentic corilagin sample (**7**) exhibited a doublet at δ 6.20 ($J = 7.2$ Hz), attributable to H-1 of the glucose moiety and indicative of the β-configuration at the anomeric centre. This finding is explicable in terms of the solvent-dependent conformational change (1C → 1B) of the glucose core (Seikel and Hillis, 1970). In contrast, the ^{1}H NMR spectrum of **8** displayed the signal of the corresponding proton as a broad singlet at δ 6.14, thus indicating an equatorial–axial arrangement of H-1 and H-2. Accordingly, compound **8** represents the unique α-isomer of **7** and was named isocorilagin.

Comparison of the ^{1}H NMR data for compound (**3**) with those for phyllanthusiin E (**2**) revealed its close structural resemblance to this phenolcarboxyclic acid. The ^{1}H NMR spectra of **2** and **3** were virtually superposable, except for the presence of an additional singlet at δ 3.72 attributable to either a methoxy function or a carboxymethyl group. This conjecture found support in the negative FAB-mass spectrum of **3**, showing a $[M - H]^-$ peak at m/z 305, in complete agreement with the expected molecular constitution. Addition of $AlCl_3$ induced a strong bathochromic shift (22 nm) in the UV spectrum relative to that recorded in methanol, indicating the presence of vicinal hydroxy groups in **3**. Collectively, these observations facilitated definition of the methyl ester arrangement for **3**. The possibility that **3** represents an artifact produced on CC with aqueous methanol was excluded by its concurrent isolation from the *n*-butanol phase that did not contain phyllanthusiin E (**2**). Thus, compound **3** was established as phyllanthusiin E methyl ester, a new ellagitannin metabolite.

Initial identification of compounds **10**–**13** as ellagitannins clearly followed from their visualisation on silica plates upon treatment with $FeCl_3$ (dark blue), potassium iodate (pink) (Haslam, 1965) and sodium nitrite/acetic acid (blue) (Bate-Smith, 1972). Diagnostic features in the ^{1}H NMR spectra of this group of hydrolysable tannins were the presence of a two-proton singlet at ca δ 7.1 for the magnetically equivalent 2- and 6-protons of a galloyl group in each instance, and aliphatic proton signals attributable to a glucose moiety (Table 1). Supporting evidence for the presence of these constructing units in **10**–**13** was available from acid hydrolysis. Location of the galloyl group at C-1 followed from a significant downfield shift of the anomeric proton in each instance, taking into account detailed shift correlation data. The chemical shifts and coupling patterns of the sugar protons which were assigned by ^{1}H–^{1}H COSY are typical of a glucopyranose residue adopting the thermodynamically less favoured $^1C_4$ conformation. These features, supported by ^{13}C NMR spectral data (Table 2), collectively established the presence of a common 1-*O*-galloyl ($^1C_4$) glucopyranose core (Tables 1 and 2) in compounds **10**–**13**. Notable differences in the ^{1}H NMR spectra of compounds **10**–**13** were associated with signals, reminiscent of those of an oxidatively modified dehydrohexa-hydroxydiphenoyl (DHHDP) entity.

The negative FAB-mass spectrum of **10** showed a $[M - H]^-$ peak at m/z 925, and the sodium complex $[M + Na]^+$ at m/z 949 in the positive mode. Analysis of its ^{1}H NMR spectrum indicated, in addition to the key features noted above, the presence of two isolated one-proton singlets at δ 6.64 and 6.82, attributable to an HHDP moiety. Close structural similarity of **10** and phyllanthusiin C (**9**) followed tentatively from the general congruence of ^{1}H and ^{13}C resonances. Notable differences included replacement of the hemiacetal absorbance (δ_C 117.3) by a ketonic carbon signal (δ_C 201.2), signifying the presence of an equivalent 'open' form. Compound **10** was, therefore, identified as a new naturally occurring ellagitannin, designated as pelargoniin A.

The absence of an HHDP moiety in compounds **11**–**13** was readily evident from 'missing' respective isolated aromatic one-proton singlets in their ^{1}H NMR spectra. This assumption was supported by three recognizable upfield-shifted signals of the $^1C_4$ glucose moiety at $\delta < 5.0$ (H-3), δ 4.03 (H-6) and ca δ 4.2 (H-6) assigned with the aid of ^{1}H–^{1}H shift correlation spectra in each instance (Table 1).

The FAB mass spectrum of **11** showed a pseudomolecular $[M - H]^-$ ion peak at m/z 621, corresponding

to the empirical formula $C_{26}H_{22}O_{18}$. Its ^{1}H NMR spectral features were closely similar to those of euphormisin M_2 (Yoshida et al., 1994), except for the absence of signals due to an HHDP residue (vide supra). In the sugar region, the signals of the anomeric proton, H-2 and H-4 appeared at significantly lower field than the remaining glucose proton signals (Table 1). This finding, taken together with the established 1-*O*-galloylation, clearly indicated acylation of the hydroxyl functions at C-2 and C-4 of the glucose core. The ^{13}C NMR spectrum of **11** indicated, in addition to signals attributable to a pyrogallol-type element, the presence of three aliphatic carbon resonances [δ_C 30.8, 44.2 and 46.0], two carbonyl carbon signals (δ_C 175.0, 175.9) and a carboxyl carbon signal (δ_C 177.1) for the 2,4-acyl moiety. Analysis of the ^{1}H NMR spectrum revealed signals for an ABXY-type pattern [δ 1.84, *dd*, $J = 2.6$ and 16.7 Hz; δ 2.15, *dd*, $J = 11.4$ and 16.7 Hz (H_2-4′), δ 3.69, *dd*, $J = 2.6$ and 11.4 Hz (H-3′), ca δ 4.95 (overlapped with solvent peak (H-2′)] in the aliphatic region. Based on these data, the structure of compound **11**, designated as pelargoniin B, was thus assigned and reported in a preliminary communication (Latté and Kolodziej, 1998). It should be noted that the natural existence of this compound was concurrently demonstrated by its isolation from *Phyllanthus virgatus* and that it was independently named virganin (Huang et al., 1998).

Compound **12** was characterized as a new ellagitannin, designated as pelargoniin C, on the basis of spectral analyses. The ^{1}H NMR spectrum of **12** showed a close resemblance to that of phyllanthusiin C (**9**), except for the above noted absence of signals due to an HHDP residue. It exhibited in the aliphatic region the presence of an AA′X-system [δ 2.24, *dd*, $J = 4.2$ and 12.0 Hz (H_a-3′); δ 2.36, *dd*, $J = 6.8$ and 12.0 Hz (H_b-3′); δ 4.68, *s* (H-1′)], besides the $^1C_4$ glucose signals (Table 1). The FAB mass spectrum of **12** displayed a $[M + H]^+$ ion peak at *m*/*z* 625, corresponding to the loss of an HHDP moiety (302 mass units) from **9** ($[M + H]^+$ at *m*/*z* 927). These spectral features collectively established the structure of pelargoniin C as **12**, which differs from **9** by the lack of the 3,6-bridging HHDP residue on the glucopyranose entity.

Compound **13** was again based on a 1-*O*-galloyl-β-D-$^1C_4$-glucopyranose precursor, as readily evident from comparing its ^{1}H NMR spectral data with those of compounds **10**–**12** (Table 1). A remarkable difference of **13** from **10**–**12** was the detection of the relative

Table 1
^{1}H NMR data (400 MHz, CD_3OD) of compounds **9**–**13** (δ in ppm from TMS, multiplicities and *J* values (Hz) are given in parentheses)[a]

	9	10	11	12	13
Glucose					
H-1	6.34 (*br s*)	6.34 (*br s*)	6.28 (*d*; $J = 2.1$,)	6.14 (*br s*)	6.36 (*br s*)
H-2	5.48 (*br s*)	5.48 (*br s*)	5.15 (*br s*)	5.29 (*br s*)	5.20 (*br s*)
H-3	5.52 (*br s*)	5.52 (*br s*)	4.97 (*br s*)	4.57 (*br s*)	4.02–5.09 (*m*)
H-4	5.37 (*br s*)	5.37 (*br s*)	[b]	[b]	
H-5	5.06 (*t*-like, $J = 10.9$,)	5.04 (*br t*, $J = 10.9$)	4.37 (*t*-like, $J = 6.3$,)	4.21 (*br t*)	4.02–5.09 (*m*)
H_a-6	4.32 (*dd*; $J = 8.3$, 10)	4.33 (*dd*; $J = 8.0$, 10.9)	4.03 (*dd*; $J = 6.8$; 11.6)	4.03 (*dd*; $J = 5.5$; 4.8)	4.02–5.09 (*m*)
H_b-6	[b]	[b]	4.15 (*dd*; $J = 6.6$; 11.6)	4.2 (*t*; $J = 7.7$)	4.02–5.09 (*m*)
Galloyl (ring A)					
H-2/ H-6	7.06 (*s*)	7.06 (*s*)	7.11 (*s*)	7.15 (*s*)	7.13 (*s*)
HHDP					
H-3	6.82 (*s*)	6.82 (*s*)	–	–	–
H-3′	6.64 (*s*)	6.64 (*s*)	–	–	–
2,4-Acyl (ring D)					
H-3	7.09 (*s*)	7.13 (*s*)	7.21 (*s*)	7.10 (*s*)	7.46 (*s*)
2,4-Acyl (ring E)					
H-1′	4.63 (*s*)	4.60 (*s*)	–	4.68 (*s*)	–
H-2′	–	–	[b]	–	3.80 (*dd*, $J = 3.6$; 11.5)
H_a-3′	2.23 (*t*-like, $J = 12.0$)	2.11 (*d*; $J = 14.0$,)	3.69 (*dd*, $J = 2.6$; 11.4)	2.24 (*dd*, $J = 4.2$; 12.0)	2.14 (*m*)
H_b-3′	2.35 (*dd*, $J = 6.8$, 12.0)	2.68 (*dd*-like, $J = 6.0$, 14.0)	–	2.36 (*dd*, $J = 6.8$; 12.0)	2.14 (*m*)
H_a-4′	4.57 (*dd*, $J = 6.8$, 10.8)	4.35 (*dd*, $J = 8.0$, 11.0)	1.84 (*dd*, $J = 2.6$; 16.7)	4.57 (*dd*, $J = 6.8$; 11.0)	–
H_b-4′	–	–	2.15 (*dd*, $J = 16.7$; 11.4)	–	–

[a] For convenience, numbering for rings A–E follows that for phyllanthusiin C (**9**) (Yoshida et al., 1992).
[b] Overlapping of signals with solvent peak.

downfield position of just the H-2 proton signal of the glucose moiety (δ 5.20, *br s*), indicating that the hydroxyl group at this position was acylated. Supporting evidence for the assignment of H-2 was available from careful analysis of two-dimensional COSY spectra recorded in both CD_3OD and acetone-d_6. For this acyloxy substituent, signals for a methylene function [δ 2.14, *m* (H-3′)], a methine proton [δ 3.80, *dd*, $J = 3.6$ and 11.5 (H-2′)], and an aromatic isolated proton [δ 7.46, *s* (ring D)] were observed in the methanol-d_4 ^{1}H NMR spectrum of **13**, suggesting the presence of an oxidatively modified DHHDP moiety. The conspicuous downfield position of the aromatic singlet [δ 7.46 (ring D)] relative to that of **9**–**12** (ca δ 7.1) was reminiscent of a lactonic carbon in close proximity to this proton (Lin et al., 1990; Hatano et al., 1992). Owing to insufficient sample quantity for ^{13}C NMR analysis and the limited information from ^{1}H NMR data, the spectrum was thus recorded in acetone-d_6 where two proton signals at δ 8.36 and 9.10 were evident, assignable to a carboxylic and an aldehyde function, respectively. Based on the above evidence, the structure of compound **13** was identified as depicted in its formula, representing another novel ellagitannin designated as pelargoniin D.

Table 2
^{13}C NMR (100 MHz, CD_3OD) spectral data of compounds **7**, **9**–**11**[a]

	7	9	10	11
Glucose				
C-1	95.1	92.6	92.7	93.3
C-2	69.5	68.3	68.5	71.8
C-3	68.5	63.0	63.1	62.3
C-4	69.5	66.0	66.4	64.5
C-5	76.2	73.4	73.5	73.4
C-6	62.5	64.5	65.8	63.8
Galloyl (ring A)				
C-1	120.6	120.0	120.2	120.7
C-2	111.1	111.0	111.0	110.5
C-3	146.2	146.5	146.6	146.7
C-4	140.4	140.8	140.9	139.9
C-5	138.2	146.5	146.6	146.7
C-6	110.5	111.0	111.0	110.5
C-7	166.7	166.0	166.2	166.5
HHDP (ring B)[b]				
C-1	116.7	117.4	117.6	–
C-2	117.2	124.4	124.5	–
C-3	108.3	110.5	110.6	–
C-4	138.2	146.3	145.6	–
C-5	137.7	138.6	138.8	–
C-6	146.4	145.7	145.6	–
C-7	168.8	167.6	167.6	–
HHDP (ring C)[b]				
C-1″	117.1	116.3	116.5	–
C-2′	116.7	125.6	125.6	–
C-3′	108.5	108.1	108.1	–
C-4′	146.1	145.7	145.6	–
C-5′	137.7	137.7	137.7	–
C-6′	146.4	146.2	146.6	–
C-7′	170.2	170.3	170.1	–
2,4-Acyl (ring D)				
C-1	–	119.3	119.1	117.5
C-2	–	117.7	117.6	117.5
C-3	–	111.9	112.3	114.6
C-4	–	147.7	147.9	148.4
C-5	–	136.5	136.4	136.8
C-6	–	150.2	150.0	143.0
C-7	–	165.8	165.5	165.7
2,4-Acyl (ring E)				
C-1′	–	63.9	64.6	177.1
C-2′	–	78.9	79.9	46.0
C-3′	–	46.9	47.0	44.2
C-4′	–	74.9	75.6	30.8
C-5′	–	117.3	201.2	175.9
C-6′	–	174.3	167.0	175.0

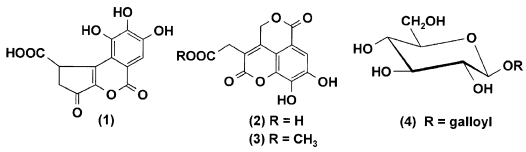
[a] For convenience, numbering for rings A–E follows that for phyllanthusiin C (**9**) (Yoshida et al., 1992).
[b] Assignments may be interchanged.

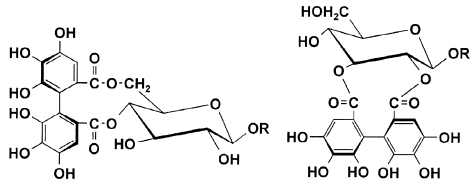
Although the principal conformation of the glucose core of analogues of this group is firmly established (Jochims et al., 1968; Seikel and Hillis, 1970; Haddock et al., 1982; Nawwar et al., 1994), the acylation patterns were found to represent an additional contributing factor towards $^1C_4$ or equivalent skew boat variations in solution. For example, the spectral features of both 3,6-, e.g. (**7**), and 2,4-monobridged members, e.g. (**11**–**13**), were consistent with a preferred 1C form in methanol, while the 1B (vide supra) and B3 form (long range coupling between H-2 and H-4) (Jochims et al., 1968) were concluded to preferentially occur in DMSO for the former and latter analogues, respectively. In contrast, the glucose moiety in compounds **9** and **10** having attached 2,4- and 3,6-acyl moieties invariably adopted the $^1C_4$ conformation in both solvents as concluded from similar coupling patterns of sugar proton signals, and the observed long range coupling between H-1 and H-3. The presence of the additional 2,4-acyl moiety, therefore, appeared to restrict the conformational flexibility.

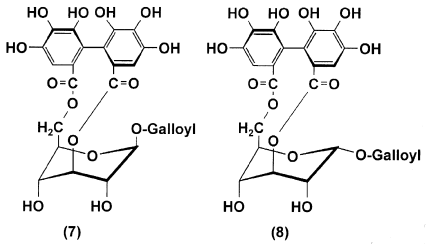
Biosynthetically, the pelargoniins isolated in the present study may be regarded as oxidative metabolites of geraniin. Thus, it is a point of some curiosity that this key intermediate has hitherto not been detected in taxa with plants producing ellagitannins possessing DHHDP moieties but the genus *Geranium*, presumably due to rapid turnover rates.

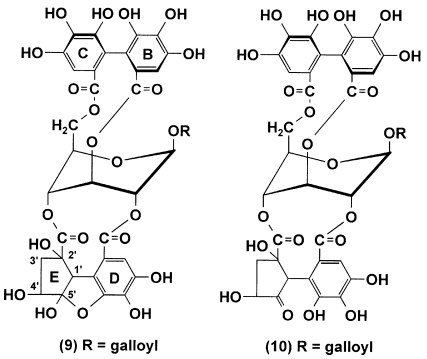
According to present evidence, ellagitannins based on a glucose core which itself adopts the less favourable $^1C_4$ conformation represent a rather small group of hydrolysable tannins with distinct occurrence in the plant kingdom (Haslam, 1998). This is the first example of an intraspecific co-occurrence of ellagitan-

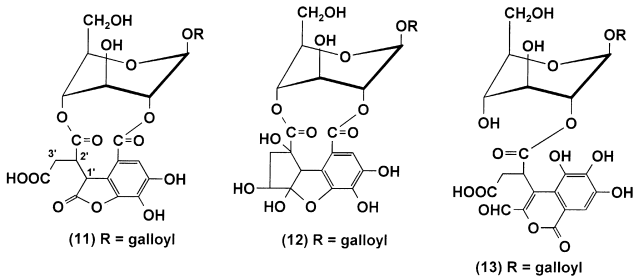
nins with both $^4C_1$ and $^1C_4$ conformation of the glucose core for a plant species of the Geraniaceae. To date, only a few plants of the Euphorbiaceae (Saijo et al., 1989a, 1989b), Melastomataceae (Yoshida et al., 1992) and Onagraceae (Haddock et al., 1982) have been shown to contain both forms of metabolites.











3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were measured with a Bruker AC-400 instrument and chemical shifts are given in δ (ppm) relative to $(Me)_4Si$. EI- and FAB-mass spectra (glycerol/xenon/DMSO) were obtained on a Finnigan MAT CA7A and a CH5DF mass spectrometer, respectively. UV spectra were recorded on a Shimadzu UV160-A model. Optical rotations were measured on a Perkin Elmer 2M/MC polarimeter. HPLC separation was done with a Knauer instrument, equipped with a gradient former and a variable UV detector, and computer integrating model (EuroChrom 2000). Experimental conditions: Eurospher 100C-18 (8 × 250 mm); mobile phase, H_2O–MeOH gradient 9:1 → 3:7 (40 min, flow rate 4 ml/min, detection at 275 nm). TLC analysis was performed on silica plates (Merck, Kieselgel $60F_{254}$; EtOAc–H_2O–HCO_2H, 18:1:1). Compounds were visualised by exposure to UV (254 nm) and by spraying $FeCl_3$, potassium iodate, and $NaNO_2$–AcOH reagents.

3.2. Plant material

The plant material of *P. reniforme* was kindly provided by Dr. Willmar Schwabe, Karlsruhe, Germany. A voucher specimen has been deposited at the Institut für Pharmazie, Freie Universität Berlin.

3.3. Extraction and isolation

The dried aerial parts of *P. reniforme* (4.1 kg) were exhaustively extracted with Me_2CO–H_2O (4:1; 70 l). The combined extracts were reduced in volume (1 l) and defatted with petroleum ether (205 × 500 ml). The aqueous phase was subsequently successively extracted with $CHCl_3$ (212 × 500 ml), EtOAc (232 × 500 ml) and *n*-butanol (50 × 500 ml). Evaporation of the solvent yielded a brown residue in each instance (9.2, 51.6 and 40.8 g, respectively).

3.3.1. Ethyl acetate phase

The ethyl acetate soluble portion (32.3 g) was initially chromatographed on Sephadex LH-20 (120 × 4 cm;) with gradient solvent systems of MeOH–H_2O (1:9–0:1) to afford 24 crude fractions, including compounds **2** and **5**–**11**. Following qualitative TLC analysis on silica gel (EtOAc–H_2O–HCO_2H, 18:1:1) appropriate fractions (15 ml) were combined and further resolved as follows:

*3.3.1.1. Phyllanthusiin E (**2**).* The content of fractions 336–455 (315 mg) was rechromatographed on Sephadex LH-20 using a MeOH–H_2O gradient system (1:19

→ 3:17), and the subfractions 43–52 were further purified by prep. HPLC to afford compound **2** (0.0008%). A white amorphous powder, R_f 0.57, R_t 14.2 min. UV λ_{max} (MeOH) nm: 224, 286, 344. FAB-MS (rel. int. %): *m/z* 291 (100) $[M - H]^-$. ^1H NMR (CD_3OD): δ 7.50 (*s*, H-2), 5.65 (2H, *s*, H_2-12), 3.54 (2H, *s*, H_2-8).

*3.3.1.2. Isostrictinin (**6**).* The content of test tubes 2101–2300 (266 mg) was similarly purified on Sephadex LH-20 with a MeOH–H_2O gradient system (1:4 → 1:1), and the subfractions 301–330 were further purified by HPLC to afford **6** (0.003%). A white amorphous powder, $[\alpha]_D^{20}$ −10.5° ($c = 0.1$ in MeOH); R_f 0.30; R_t 10.7 min. UV λ_{max} (MeOH) nm: 225, 268.; FAB-MS (rel. int. %): *m/z* 633 (100) $[M - H]^-$, 657 (100) $[M + Na]^+$. ^1H NMR (CD_3OD): δ 7.09 (2H, *s*, Galloyl H-2/H-6), 6.66 (1H, *s*, HHDP H-3), 6.37 (1H, *s*, HHDP H-3′), 6.08 (1H, *d*, $J = 8.4$, Glc H-1), 5.19 (1H, *t*, $J = 9.5$, Glc H-3), 5.08 (1H, *t*, $J = 8.6$, 9.5, Glc H-2), 3.53–3.93 (4H, *m*, Glc H-4–H_2-6). Physical data corresponded to those in the literature (Tanaka et al., 1985; Okuda et al., 1982b).

3.3.1.3. Corilagin (7). Chromatography of the subfractions 213–235 on Sephadex LH-20, followed by HPLC purification as noted above afforded **7** (0.11 %). A white amorphous powder, $[\alpha]_D^{20}$ −250.0° ($c = 0.3$ in MeOH), R_f 0.51, R_t 9.9 min. UV λ_{max} (MeOH) nm: 219, 270. FAB-MS (rel. int. %): *m/z* 633 $[M - H]^-$ (100), 657 (30) $[M + Na]^+$. CD $[\Theta]_{243}$ −94,000, $[\Theta]_{270}$ +29,000, $[\Theta]_{296}$ −30,000. ^1H and ^{13}C NMR data corresponded to those in the literature (Nawwar et al., 1994).

*3.3.1.4. Phyllanthusiin C (**9**).* The content of test tubes 2601–2960 was subjected to HPLC purification to yield **9** (0.035%). A white amorphous powder, mp. 214°C, $[\alpha]_D^{20}$ −87.0° ($c = 0.1$ in MeOH), R_f 0.35, R_t 16.7 min. UV λ_{max} (MeOH) nm: 223, 278. FAB-MS (rel. int. %): *m/z* 949 (4) $[M + Na]^+$, 925 (20) $[M - H]^-$. ^1H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2.

*3.3.1.5. Pelargoniin A (**10**).* HPLC purification of the subfractions 296–400, obtained from chromatography on Sephadex LH 20 of the fractions 2601–2960, afforded **10** (0.0075 %). A white amorphous powder, mp. 220°C, $[\alpha]_D^{20}$ −65.5° ($c = 0.06$ in MeOH), R_f 0.15, R_t 18.5 min. UV λ_{max} (MeOH) nm: 223, 280. FAB-MS (rel. int. %): *m/z* 925 (100) $[M - H]^-$, 949 (100) $[M + Na]^+$. ^1H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2.

*3.3.1.6. Pelargoniin B (**11**).* A white amorphous powder from subfractions 296–400 (0.017%), R_f 0.41, R_t 17.6 min. Mp. 214°C, $[\alpha]_D^{20}$ −43.0° ($c = 0.5$ in MeOH). UV λ_{max} (MeOH) nm: 225, 268. FAB-MS (rel. int. %): *m/z* 621 (8) $[M - H]^-$. ^1H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2.

*3.3.1.7. Pelargoniin D (**13**).* The content of test tubes 1901–2100 was subjected to HPLC purification to yield **13** (0.0013%). A white amorphous powder, R_f 0.41, R_t 7.1 min. UV λ_{max} (MeOH) nm: 225, 268. FAB-MS (rel. int. %): *m/z* 651 (100) $[M - H]^-$, 675 (100) $[M + Na]^+$. ^1H NMR (CD_3OD): see Table 1.

3.3.2. Butanol phase

The *n*-butanol soluble portion (17.4 g) was similarly separated on Sephadex LH-20 with gradient solvent systems of MeOH–H_2O (1:9–0:1) to afford 14 crude fractions. Subsequent HPLC separation of distinct subfractions led to the isolation of compounds **1**, **3**–**5**, **8** and **12**.

*3.3.2.1. Brevifolincarboxylic acid (**1**).* The content of test tubes 351–515 (63 mg) was subjected to HPLC separation to afford **3** (0.026%). A yellow amorphous powder, R_f 0.59, R_t 13.0 min, $[\alpha]_D^{20}$ −18.3° ($c = 0.9$ in acetone). UV λ_{max} (MeOH) nm: 212, 280, 348. FAB-MS (rel. int. %): *m/z* 291 (100) $[M - H]^-$. ^1H and ^{13}C NMR data identical to those in the literature (Yoshida et al., 1992).

*3.3.2.2. Phyllanthusiin E methyl ester (**3**).* The content of test tubes 271–350 (280 mg) was rechromatographed on Sephadex LH-20 (120 × 2.5 cm) eluting with a gradient system of MeOH–H_2O (5:95 → 15:85). Subfractions 221–260 were further purified by prep. HPLC to afford compound **3** (0.0011%). A white amorphous powder, R_f 0.83, R_t 7.3 min. Mp. 158–160°C. UV λ_{max} (MeOH) nm: 228; 278; 340; +$AlCl_3$: 225, 296, 366. FAB-MS (rel. int. %): *m/z* 305 (100) $[M - H]^-$. ^1H NMR (CD_3OD): δ 7.51 (*s*, H-2), 5.65 (2H, *s*, H_2-12), 3.72 (3H, OCH_3), 3.60 (2H, *s*, H_2-8).

*3.3.2.3. 1-O-Galloyl-β-D-glucopyranosid (**4**).* The content of test tubes 146–270 (77 mg) were subjected to HPLC purification to yield **4** (0.017%). A white amorphous powder, R_f 0.20, R_t 2.5 min, $[\alpha]_D^{20}$ −8.0° ($c = 0.1$ in MeOH). FAB-MS (rel. int. %): *m/z* 331 (90) $[M - H]^-$. ^1H NMR (CD_3OD): δ 7.13 (2H, *s*, Galloyl H-2/H-6), 5.65 (1H, *d*, $J = 7.8$, Glc H-1), 3.85 (1H, *dd*, $J = 12.4$, 1.1 Glc H_b-6), 3.70 (1H, *dd*, $J = 12.4$, 5.8, Glc H_a-6), 3.41–3.48 (4H, *m*, Glc H-2–H-5). ^1H NMR data (acetone-d_6) are consistent with those in the literature (Kashiwada et al., 1984).

*3.3.2.4. Strictinin (**5**).* The content of test tubes 1601–1840 (60 mg) was subjected to HPLC separation to afford **5**. A white amorphous powder, R_f 0.20, R_t 3.6 min, $[\alpha]_D^{20}$ −2.9° ($c = 0.1$ in MeOH). UV λ_{max} (MeOH)

nm: 219, 275. FAB-MS (rel. int. %): *m/z* 657 (100) [M + Na]$^+$, 633 (100) [M − H]$^-$. Physical data are consistent with those in the literature (Nonaka et al., 1984).

*3.3.2.5. Isocorilagin (**8**).* The content of test tubes 941–1390 (330 mg) was rechromatographed on Sephadex LH-20 and the subfractions 351–440 subjected to HPLC separation to afford **8** (0.006%): R_f 30, R_t 8.3 min; $[\alpha]_D^{20}$ −53.6° ($c = 0.11$ in MeOH). FAB-MS (rel. int. %): *m/z* 633 (100) [M − H]$^-$. ^{1}H NMR (CD_3OD): δ 7.05 (2H, *s*, Galloyl H-2/H-6), 6.68 (1H, *s*, HHDP H-3), 6.65 (1H, *s*, HHDP H-3′), 6.36 (1H, *d*, $J = 1.7$, Glc H-1), 5.00 (1H, overlapped by solvent peak, Glc H-5), 4.80 (1H, *br s*, Glc H-3), 4.51 (1H, *dd*, $J = 10.9$, 9.0, Glc H_a-6), 4.46 (1H, *d*, $J = 3.0$, Glc H-4), 4.15 (1H, *dd*, $J = 8.1$, 10.9, Glc H_b-6), 3.98 (1H, *br s*, Glc H-2). CD $[\Theta]_{246}$ −11,000, $[\Theta]_{271}$ +5500, $[\Theta]_{293}$ −4100.

*3.3.2.6. Pelargoniin C (**12**).* The content of test tubes 941–1390 (330 mg) was rechromatographed on Sephadex LH-20 and the subfractions 261–333 subjected to HPLC separation to afford 12 (0.005%). A white amorphous powder, mp. 210°C. UV λ_{max} (MeOH) nm: 224, 284. R_f: 0.30; R_t: 6.9 min. FAB-MS (rel. int. %): *m/z* 647 (100) [M + Na]$^+$, 625 (100) [M + H]$^+$. ^{1}H NMR (CD_3OD): see Table 1.

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