PERGAMON

Phytochemistry 59 (2002) 419–424

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

O-Galloyl-*C*-glycosylflavones from *Pelargonium reniforme*

Klaus Peter Latté[a], Daneel Ferreira[b], M.S. Venkatraman[c], Herbert Kolodziej[a,*]

[a]*Institut für Pharmazie, Pharmazeutische Biologie, Freie Universität Berlin, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany*
[b]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*
[c]*Department of Medicinal Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

Received 12 July 2001; received in revised form 31 August 2001

Abstract

The unique series of C-2″-acylated *C*-glycosylflavones is extended by the discovery of the C-8-glucosyl derivatives 2″-*O*-galloylvitexin and 2″-*O*-galloylorientin and their C-6 analogues 2″-*O*-galloylisovitexin and 2″-*O*-galloylisoorientin, representing the first described *O*-galloyl-C-glycosylflavones. They are accompanied in the aerial parts of *Pelargonium reniforme* by the known non-galloylated parent analogues vitexin, orientin, isovitexin and isoorientin, as well as several known flavonoid-*O*-glycosides. The structures of these compounds were established from spectroscopic studies. Differentiation between *C*-glycosylation at C-6 and C-8 is discussed on the basis of the effects of dynamic rotational isomerism.

Keywords: *Pelargonium reniforme*; Geraniaceae; *C*-Glycosylflavones; 2″-*O*-Galloylvitexin; 2″-*O*-Galloylorientin; 2″-*O*-Galloylisovitexin; 2″-*O*-Galloylisoorientin; Flavonoids

1. Introduction

The genus *Pelargonium* comprises more than 250 species of perennial small shrubs which are limited in their geographical distribution. About 80% of these species are confined to the southern parts of Africa, while others occur in Australia, New Zealand and in the Far East (Van der Walt and Vorster, 1983). The importance of *Pelargonium* plants in traditional medicine is well documented (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996). For example, *Pelargonium reniforme* CURT. is highly estimated by traditional practitioners for its curative properties. The therapeutic significance of this plant is also documented by its present utilisation in modern phytotherapy (Heil and Reitermann, 1994; König, 1995). Interest in this plant has been increased by reports of antituberculotic activities and its potential as a remedy for respiratory tract infections (Helmstädter, 1996).

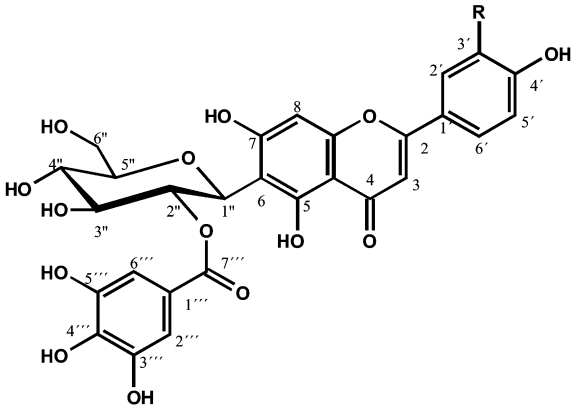
In order to provide a chemical rationale for its popularity in traditional medicine, a detailed investigation of *P. reniforme* was initiated. In earlier papers, we reported on the composition of the essential oil (Kayser et al., 1998) and on the structural elucidation of hydrolysable tannins (Latté and Kolodziej, 2000a) and coumarins (Latté et al., 2000). Here, the isolation and structural elucidation of naturally occurring *O*-galloyl-*C*-glycosylflavones (**1**–**4**), associated with their non-galloylated parent analogues (**5**–**8**), and the flavonoid patterns of the roots and the aerial parts of *P. reniforme* are reported for the first time. These include known *O*-glycosides based on the flavonols kaempferol, quercetin and myricetin (**9**–**16**), the flavone luteolin (**17**), the flavanone naringenin (**18**), and the dihydroflavonol (+)-taxifolin (**19**) together with the aglycones **20**–**21**.

2. Results and discussion

The individual aqueous acetone extracts of roots, herbal parts and flowers of *P. reniforme* were successively treated with petroleum ether, chloroform, ethyl acetate and *n*-butanol. The ethyl acetate soluble portions were separately subjected to repeated chromatography alternating between Sephadex LH-20 and RP-18 material using water-methanol gradients to afford a complex mixture of flavonoids comprising a series of

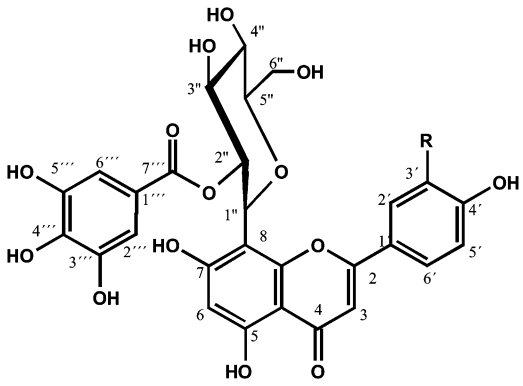
* Corresponding author. Tel.: +30-838-53731; fax: 30-838-53729.
E-mail address: kolpharm@zedat.fu-berlin.de (H. Kolodziej).

0031-9422/02/$ - see front matter
PII: S0031-9422(01)00403-4



1 R = H

2 R = OH



3 R = H

4 R = OH

dihydroflavonols, flavonols, a flavanone and a flavone (Table 1). Besides the presence of common *O*-glycosides (**9**–**19**) and aglycones (**20**–**21**), the extract also yielded a series of C–C linked β-D-glucopyranosides (**1**–**8**) based on the flavones, apigenin and luteolin, including a unique series of 2″-galloyl analogues (**1**–**4**). The latter four compounds represent the first reported naturally occurring *O*-galloyl derivatives of *C*-glycosylflavones.

Compound **1** was obtained as a yellow amorphous powder, $[\alpha]_D^{20}$ −165° ($c = 0.07$ in methanol). It was characterized as a flavone derivative on the basis of its UV absorption (λ_{max} 366 nm) and the presence of the characteristic isolated C-3 one-proton signal at δ 6.51 in its ^{1}H NMR spectrum. The FAB mass spectrum of **1** showed an $[M+H]^+$ peak at m/z 585, and the [M-galloyl-H]$^-$ ion at m/z 431 in the negative mode. Analysis of the ^{1}H NMR spectrum of **1** revealed the presence of an additional isolated one-proton singlet at δ 6.39 (8-H), an aromatic A_2B_2-spin system for the B-ring, and aliphatic proton signals arising from a β-D-glucopyranosyl moiety (Table 2) ($J_{1'',\,2''} = 10.0$ Hz), analogous to those of isovitexin (**5**) (Maatooq et al., 1997). Location of the glycosyl residue at C-6 clearly followed from a significant downfield shift ($\Delta\delta$ −10 ppm) of the respective ^{13}C resonance appearing at δ 109.3 when compared to that of the parent aglycone apigenin (Shen et al., 1993). Notable features in the ^{1}H NMR spectrum of compound **1** were the appearance of a sharp low-field two-proton singlet at δ 6.93 for the magnetically equivalent 2- and 6-protons of a galloyl group and the conspicuous deshielding of 2″-H of the glycosyl residue ($\Delta\delta$ −1.61 ppm) relative to its chemical shift in the non-galloylated analogue isovitexin (**5**), indicating that the hydroxyl group at this position was acylated. Supporting evidence for the presence of a galloyl group was also available from the characteristic fragment at m/z 170 in the EI mass spectrum, while the galloylation at C-2″ was confirmed by comparison of the ^{13}C NMR data of (**1**) with those of isovitexin (**5**), indicating a downfield shift of $\Delta\delta$ 2.1 ppm for the *ipso* carbon and shielding of $\Delta\delta$ 1.1 and 2.1 ppm of carbons C-1″ and C-3″, respectively. These spectral features established the constitution of the new 2″-*O*-galloylisovitexin (**1**).

The close structural relationship between compounds **1** and **2** was evident from similar spectral features. The most significant difference between the ^{1}H NMR spectra of compounds **1** and **2** was the replacement of the A_2B_2-spin system by an ABX-type pattern in that of **2**. Taking into account the $[M+H]^+$ peak at m/z 601 in the positive

Table 1
Flavonoids isolated from *Pelargonium reniforme* (percentage related to dry weight)

No.	Compound	Herb	Flowers	Root
1	2″-*O*-Galloylisovitexin	0.04	–	–
2	2″-*O*-Galloylisoorientin	0.04	–	–
3	2″-*O*-Galloylvitexin	0.04	–	–
4	2″-*O*-Galloylorientin	0.03	–	–
5	Isovitexin	0.35	–	–
6	Isoorientin	0.08	0.35	–
7	Vitexin	0.04	–	–
8	Orientin	0.11	–	–
9	Kaempferol 7-*O*-β-D-glucopyranoside	0.01	0.38	–
10	Kaempferol 3-*O*-β-D-glucopyranoside	–	–	0.003
11	Kaempferol 3-*O*-β-D-galactopyranoside	–	–	0.001
12	Kaempferol 3-*O*-β-rutinoside	–	0.13	–
13	Quercetin 7-*O*-β-D-glucopyranoside	0.002	0.44	–
14	Quercetin 3-*O*-β-D-glucopyranoside	–		0.008
15	Rutin	0.008	0.03	–
16	Myricetin 3-*O*-β-D-glucopyranoside	–		0.004
17	Luteolin 7-*O*-β-D-glucopyranoside	0.001		–
18	Naringenin 7-*O*-β-D-glucopyranoside	0.003	0.10	–
19	Taxifolin 7-*O*-β-D-glucopyranoside	–	0.48	–
20	(+)-Taxifolin	–	0.12	–
21	(+)-Dihydrokaempferol	–	0.12	–

Table 2
^{1}H NMR spectral data (400 MHz, CD_3OD) of the *O*-galloyl-*C*-glycosylflavones **1**–**4** [δ in ppm from TMS, multiplicities and *J* values (Hz) are given in parentheses]

	1	**2**	**3**	**4**
Proton				
H-3	6.51 (*s*)	6.45 (*s*)	6.61, 6.41[a] (*s*)	6.55, 6.37[a] (*s*)
H-6	–	–	6.07, 6.22[a] (*s*)	6.6, 6.21[a] (*s*)
H-8	6.39 (*s*)	6.39 (*s*)	–	–
H-2′	7.79 (*d*; 8.7)	7.34 (*d*; 2.0)	8.07, 7.82[a] (*d*; 8.7)	7.68, 7.38[a] (*d*; 1.9)
H-3′	6.89 (*d*; 8.7)	-	6.97 (*d*; 8.7)	–
H-5′	6.89 (*d*; 8.7)	6.89 (*d*; 8.9)	6.97 (*d*; 8.7)	6.97 (*d*; 8.4)
H-6′	7.79 (*d*; 8.7)	7.35 (*dd*; 8.9, 2.0)	8.07, 7.82[a] (*d*; 8.7)	7.61, 7.40[a] (*dd*; 8.4, 1.9)
Glucosyl				
H-1″	5.18 (*d*; $J=10.0$)	5.16 (*d*; $J=10.0$)	5.19, 5.32[a] (*d*; 10.1)	5.22, 5.35[a] (*d*; 10.1)
H-2″	5.77 (*br t*)	5.72 (*br t*)	5.71, 5.62[a] (*t*; 9.6)	5.74, 5.66[a] (*t*; 9.7)
H-3″	3.75 (*t*; 9.4, 9.1)	3.76 (*t*; 9.6)	3.83 (*t*; 9.4)	3.83 (*t*; 9.0)
H-4″	3.62 (*t*; 9.4)	3.61 (*t*; 9.4, 9.1)	3.76 (*t*; 9.4)	3.79 (*t*; 9.0)
H-5″	3.48 (*m*)	3.50 (*m*)	3.55 (*m*)	3.55 (*m*)
H_a-6″	3.92 (*dd*; 12.0, 2.0)	3.93 (*dd*; 12.0, 1.8)	4.01 (*dd*; 1.8, 12.0)	4.02 (*dd*; 1.9, 12.1)
H_b-6″	3.78 (*dd*; 12.0, 5.4)	3.79 (*dd*; 5.5, 12.0)	3.84 (*dd*; 5.7, 12.0)	3.91 (*dd*; 5.8, 12.1)
Galloyl				
H-2‴	6.93 (*s*)	6.93 (*s*)	6.89, 6.68[a] (*s*)	6.89, 6.69[a] (*s*)
H-6‴	6.93 (*s*)	6.93 (*s*)	6.89, 6.68[a] (*s*)	6.89, 6.69[a] (*s*)

[a] δ-Value of the minor rotamer.

FAB mass spectrum, compound **2** was identified as 2″-*O*-galloylisoorientin, representing another new *O*-galloylated *C*-glycosylflavone.

Although C-6 substituted *C*-glycosylflavonoids are reported to show no evidence of rotational isomerism about the sp^2 C→anomeric carbon bond, it is noteworthy that the ^{1}H NMR spectra of compounds **1** and **2** at room temperatures displayed severe line broadening of the well separated 2″-H signal of the glycosyl unit in each instance. Owing to the overlap of signals, similar effects for other protons were less discernable. Such a phenomenon presumably reflected restricted conformational flexibility at the C_6–$C_{1''}$ bond (see below) associated with the presence of the 2″-*O*-galloyl group, as has been shown for the dynamic rotational isomerism of phenolic methyl ether *O*-acetyl derivatives of isovitexin (Rabe et al., 1994).

FAB mass spectral analysis of compounds **3** and **4** showed again the quasimolecular $[M+H]^+$ ions at *m*/*z* 585 and 601, respectively, corresponding to the molecular formulae $C_{28}H_{24}O_{14}$ and $C_{28}H_{24}O_{15}$. Again, the key features of the ^{1}H NMR spectra of **3** and **4** were two isolated singlets for the 3-H and the 'residual' aromatic A-ring proton, a low-field two-proton signal for a galloyl group and aliphatic proton signals attributable to a β-D-glycopyranosyl moiety ($J_{1'',2''}=10.1$ Hz) in each instance, and the aromatic A_2B_2- and the ABX-spin systems for the B-rings of **3** and **4**, respectively (Table 3). Their ^{1}H NMR spectra at ambient temperatures, however, displayed the typical duplication of signals that characterizes the spectra of flavones and flavonols containing a C-8-hexosyl substituent (Bezuidenhoudt et al., 1987; Markham et al., 1987; Rabe et al., 1994). The presence of isomers was also reflected by two sets of signals in the corresponding ^{13}C NMR spectra, though some of the ^{13}C resonances attributable to the minor rotamer appeared to be obscured in the base-line. Accordingly, compounds **3** and **4** represented galloylated derivatives of vitexin (**7**) and orientin (**8**), respectively. Location of the acyl moiety at C-2″ in **3** and **4** was evident from the pronounced deshielding of 2″-H ($\Delta\delta$ ca 1.1 ppm) when compared to their parent non-galloylated analogues, as well as ^{13}C chemical shift differences ($\Delta\delta$ ca +4.1, −0.7 and +3.8 ppm for C-1″, C-2″ and C-3″, respectively) compatible with acylation at C-2″ of the carbohydrate moiety (vide supra). These unambiguously defined **3** and **4** as the new *C*-glycosylflavones, 2″-*O*-galloylvitexin and 2″-*O*-galloylorientin, respectively.

The two rotamers of **3** and **4** coexisted in the ratio of 8:1 in each instance, as judged from integral intensity. Interestingly, the ratio of rotamer populations is apparently strongly solvent-dependent. With acetone-d_6 as solvent, signal intensity of the minor rotamer was enhanced, culminating in a ca 3:1 relative abundance of the two conformers.

Following demonstration of the effects of dynamic rotational isomerism in spectra of C-8 glycosylflavonoids and absence of similar phenomena in C-6 substituted analogues (Markham et al., 1987; Rabe et al., 1994), some remarkable features emerged from this investigation. Analysis of ^{1}H NMR spectra of the C-8 substituted

Table 3
^{13}C NMR spectral data (100 MHz, CD_3OD) of compounds **1**–**4**

	1	2	3	4
Carbon				
C-2	166.6	166.6	166.6	166.6
C-3	102.9	102.9	102.7	102.7
C-4	182.9	182.9	183.1	183.1
C-5	161.7	163.9	161.7	161.7
C-6	109.3	109.3	98.2	98.1
C-7	164.0	163.9	163.3	163.3
C-8	95.0	95.0	104.6	104.6
C-9	157.8	157.8	157.0	157.0
C-10	103.9	103.9	102.7	104.6
C-1′	122.2	122.6	122.0	122.0
C-2′	128.5	113.2	129.1	114.0
C-3′	115.9	145.9	115.9	145.9
C-4′	161.7	149.9	161.7	149.9
C-5′	115.9	115.7	115.9	115.7
C-6′	128.5	119.3	129.1	119.9
Glucosyl				
C-1″	72.2	72.2	71.4	71.4
C-2″	72.9	72.9	73.1	73.0
C-3″	77.1	77.2	77.0	77.1
C-4″	70.8	70.8	71.5	71.4
C-5″	81.9	81.9	82.2	82.2
C-6″	61.8	61.8	61.6	61.8
Galloyl				
C-1‴	120.5	120.5	119.9	119.9
C-2‴	109.3	109.3	109.3	109.2
C-3‴	145.2	145.2	145.2	145.2
C-4‴	138.6	138.6	138.6	138.6
C-5‴	145.2	145.2	145.2	145.2
C-6‴	109.3	109.3	109.3	109.2
C-7‴	165.7	165.7	165.7	165.7

analogues vitexin (**7**) and orientin (**8**) revealed the common phenomenon of rotational isomerism, as evidenced by the selective line-broadening of signals attributable to the 2- and 6-H of the B-ring and the C-6 hydroxymethyl function of the glycosyl residue, but absence of signal anomalies in the ^{1}H NMR spectra of their C-6 substituted analogues isovitexin (**5**) and isoorietin (**6**). This dynamic behaviour was consistent with steric interactions between these functionalities, characteristic of 8-glucosylated derivatives (Markham et al., 1987; Maatooq et al., 1997). A more complex situation was observed for the galloylated analogues **3** and **4** where two preferred conformations (vide infra) are apparently present under the experimental conditions employed (MeOH-d_4, room temperature), hence culminating in the duplication of all ^{1}H and ^{13}C resonances. By contrast, no doubling of signals has been found with vitexin 2″-rhamnoside (Markham et al., 1987). It has been suggested that the presence of an additional sugar at the 2″-position apparently locks the 8-glucosyl unit in a position that precludes its interaction with the aromatic B-ring. Such a difference presumably results from the dissimilar capabilities of the 2″-rhamnosyl and galloyl groups for spatial interaction with adjacent functional groups, especially the B-ring. This finding might be of potential value in the structure elucidation of *C*-glycosyl flavonoids containing an acylated C-8 linked hexosyl residue. It should furthermore be considered in conjunction with the selective line-broadening of the 2″-H signal in the ^{1}H NMR spectra of the C-6 isomers **1** and **2**. These issues are discussed in more detail below.

The isolation of compounds **1**–**4** not only extends the range of natural *C*-glycosylflavones but also introduces the first examples of *O*-galloyl derivatives in this group of secondary products. C-8-glycosylflavones with their 2″-acyl functionality represent a relatively rare group of naturally occurring metabolites, being hitherto limited to 2″-*p*-coumaroylvitexin (Sood et al., 1976), 2″-*p*-coumaroylvitexin 7-*O*-glucoside (Chopin et al., 1984), and 2″-caffeoylorientin (Leitao and Monache, 1998). However, none of these authors reported any evidence of rotational isomerism. Lowest energy conformations **22** and **23** (Fig. 1) for the isovitexin- and isoorientin-2″-*O*-gallates **1** and **2**, respectively, and **24** and **25** (Fig. 1) for the vitexin- and orientin-2″-*O*-gallates **3** and **4**, respectively were found with a Monte Carlo style conformational search using the MM2 force field (Mohamadi et al., 1990). These indicated the pronounced influence of the aromatic ring of the gallate moiety on the conformational itinerary about the C-6 and C-8→glycosyl bonds. In the C-6 glycosylated compounds **1** and **2** the preferred conformations **22** and **23** are those that are stabilised via attractive π-stacking (Hunter and Sanders, 1990; Steynberg et al., 1991) between the A-ring and the galloyl aromatic ring. This conformation is obviously stable enough to retard free rotation about the C-6-glycosyl bond to such an extent that broadened ^{1}H NMR resonances are observed. In the C-8 glycosylated analogues **3** and **4** the preferred conformations **24** and **25** are stabilised by π-stacking between the aromatic ring of the ester and the B-ring. Owing to the almost perfect alignment of these two π-systems this interaction will exercise a more pronounced stabilising effect than the corresponding stacking between the aromatic rings in the C-6 coupled isomers **1** and **2**. Rotation will thus be slowed down on the NMR time-scale to such an extent that two distinct rotamers are observed, hence resulting in duplication of all ^{1}H and ^{13}C NMR resonances. The absence of duplicated resonances in the ^{1}H NMR spectrum of vitexin 2″-rhamnoside (Markham et al., 1987) may thus be explained in terms of the inability of the rhamnosyl moiety to π-stack with the B-ring.

The flavonoid pattern of *P. reniforme* (Table 1) is in accord with its taxonomic grouping in section *Reniformia* based on chemical characters (Dreyer et al., 1992; Williams et al., 2000). The occurrence of *C*-glycosylflavones is of chemotaxonomic significance as these metabolites are apparently confined to distinct sections of the genus *Pelargonium* (Williams et al., 2000).

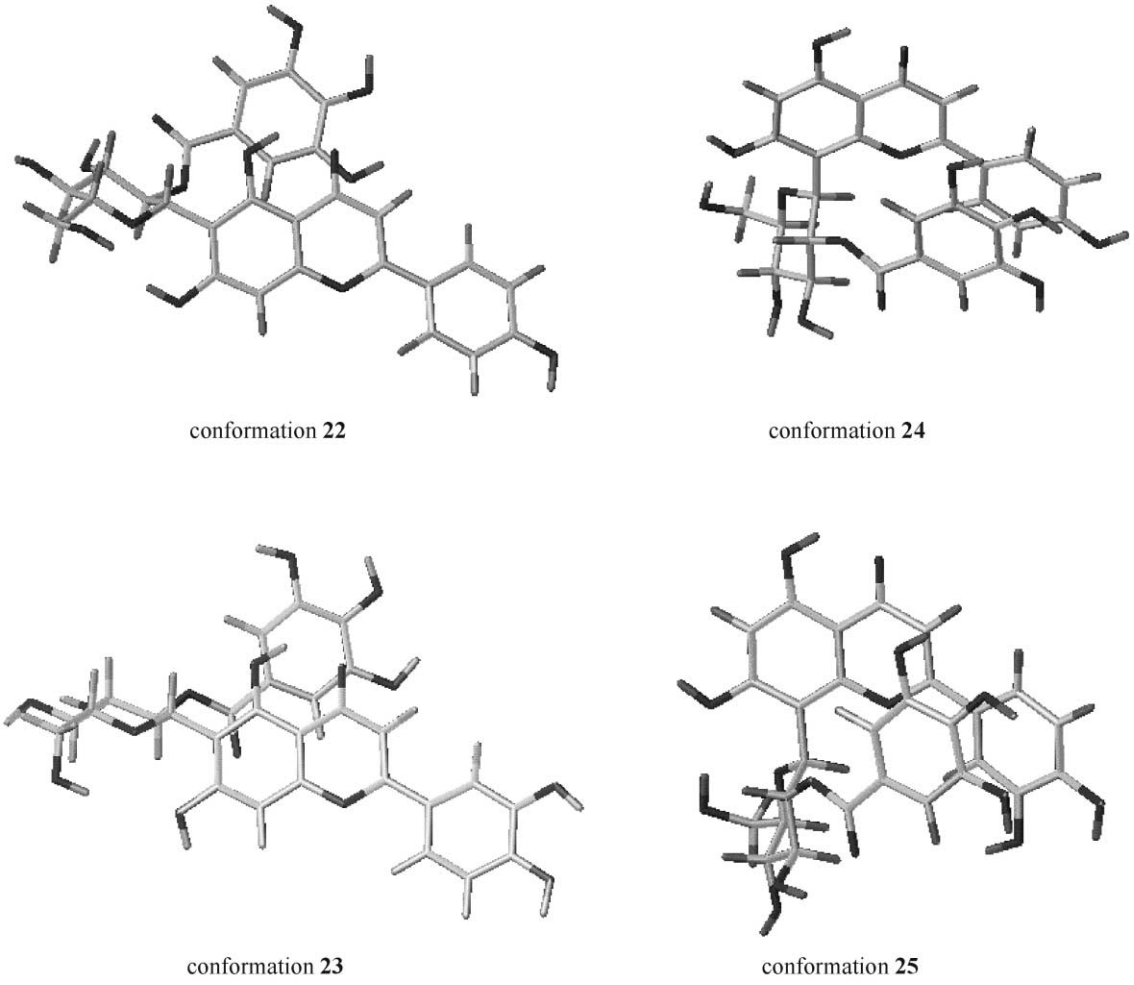


Fig. 1. Low energy conformations (**22**–**25**) of the isolated *O*-galloyl-C-glycosylflavones **1**–**4,** respectively, as found by a Monte Carlo style conformational search using the MM2 force field.

3. Experimental

3.1. General

General experimental procedures and origin of the plant material of *P. reniforme* are described in a recent publication (Latté and Kolodziej, 2000). TLC analysis was performed on silica plates (Merck, Kieselgel $60F_{254}$; EtOAc–H_2O–HCO_2H, 18:1:1). Compounds were viewed in UV light and visualized by spraying with Naturstoff-reagenz A to enhance spot detection.

3.2. Extraction and isolation

The dried parts of *P. reniforme* (roots: 1.5 kg; aerial parts: 4.1 kg; floral parts: 10.3 g) were exhaustively extracted with Me_2CO–H_2O (4:1). The respective combined extracts were reduced in volume and lipids were extracted with petroleum ether. The aqueous phase was subsequently successively extracted with $CHCl_3$, EtOAc and *n*-butanol.

3.2.1. Roots

The EtOAc-soluble portion (10 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 several crude fractions. Subsequent HPLC separation of distinct subfractions led to the isolation of compounds **10**, **11**, **14** and **16**.

3.2.2. Aerial parts

The EtOAc extractives of the aerial parts (17.4 g) were similarly subjected to chromatography on Sephadex LH-20 and HPLC separations to afford compounds **1**–**9**, **13**, **15**, **17**, **18**, **20**.

*3.2.2.1. 2″-O-Galloylisovitexin (**1**).* The content of test tubes 2961–3280 (60 mg), obtained from chromato-

graphy on Sephadex LH-20, was further purified by prep. HPLC to afford compound **1**, a yellow amorphous powder, $[\alpha]_D^{20}$ −165° ($c = 0.07$, methanol). UV λ_{max} (MeOH) nm: 221, 271, 334. R_f: 0.37; R_t: 17.6 min; pos. FAB–MS (rel. int.%): m/z 585 (100) $[M+H]^+$; neg. FAB–MS (rel. int.%): m/z 583 (100) $[M-H]^-$; m/z 431 (66) [M-galloyl-H]−. ^{1}H NMR (CD_3OD): see Table 2. ^{13}C NMR (CD_3OD): see Table 3.

*3.2.2.2. 2″-O-Galloylisoorientin (**2**).* Purification of the same subfraction (test tubes 2961–3280; 60 mg) by prep. HPLC afforded compound **2** at R_t 14.8 min. A yellow amorphous powder, $[\alpha]_D^{20}$ −174° ($c = 0.13$, methanol). UV λ_{max} (MeOH) nm: 222, 270, 346. R_f: 0.31; pos. FAB–MS (rel. int.%): m/z 601 (100) $[M+H]^+$; neg. FAB–MS (rel. int.%): m/z 599 (3) $[M-H]^-$, m/z 447 (37) [M-galloyl-H]$^-$, m/z 169 (67) [gallic acid-H]$^-$. ^{1}H NMR (CD_3OD): see Table 2. ^{13}C NMR (CD_3OD): see Table 3.

*3.2.2.3. 2″-O-Galloylvitexin (**3**).* The content of test tubes 2601–2960 (78 mg) was further purified by prep. HPLC to afford compound **3**. A yellow amorphous powder, $[\alpha]_D^{20}$ −235.5° ($c = 0.11$, methanol). UV λ_{max} (MeOH) nm: 226, 268, 333. R_f: 0.37; R_t: 24.1 min; pos. FAB–MS (rel. int.%): m/z 585 (100) $[M+H]^+$; neg. FAB–MS (rel. int.%): m/z 583 (56) $[M-H]^-$, m/z 431 (28) [M-galloyl-H]$^-$, m/z 169 (67) [gallic acid-H]$^-$. ^{1}H NMR (CD_3OD): see Table 2. ^{13}C NMR (CD_3OD): see Table 3.

*3.2.2.4. 2″O-Galloylorientin (**4**).* The content of test tubes 2601–2960 (78 mg) was similarly purified by prep. HPLC to afford compound **4**. A yellow amorphous powder, $[\alpha]_D^{20}$ −228.7° ($c = 0.15$, methanol). UV λ_{max} (MeOH) nm: 223, 270, 346. R_f: 0.47; R_t: 22.2 min; pos. FAB–MS (rel. int.%): m/z 601 (100) $[M+H]^+$; neg. FAB–MS (rel. int.%): m/z 599 (100) $[M-H]^-$, m/z 447 (25) [M-galloyl-H]$^-$ m/z 169 (67) [gallic acid-H]$^-$. ^{1}H NMR (CD_3OD): see Table 2. ^{13}C NMR (CD_3OD): see Table 3.

3.2.3. Floral parts

The EtOAc-soluble portion (1.2 g) was initially similarly subjected to CC on Sephadex LH-20 (MeOH–H_2O 1:19→0:1). Following qualitative TLC analysis on silica gel (vide supra) appropriate fractions (15 ml) were combined and further resolved by repeated chromatography in combination with HPLC separations to afford compounds **6**, **9**, **12**, **13**, **15** and **18**–**21**.

Acknowledgements

This work was supported in part by the US Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement No. 58-6408-7-012.

References

Bezuidenhoudt, B.C.B., Brandt, E.V., Ferreira, D., 1987. Flavonoid analogues from *Pterocarpus* species. Phytochemistry 26, 531–535.

Chopin, J., Dellamonica, G., Markham, K.R., Ramachandra Nair, A.G., Guansegaran, R., 1984. 2″-*p*-Coumaroylvitexin 7-glucoside from *Mollugo oppositifolia*. Phytochemistry 23, 2106–2108.

Dreyer, L.L., Albers, F., van der Walt, J.J.A., Marschewski, D.E., 1992. Subdivision of *Pelargoniu* sect *Cortusina* (Geraniaceae). Plant Systematics and Evolution 183, 83–97.

Heil, C., Reitermann, U., 1994. Atemwegs- und HNO-Infektionen. Therapeutische Erfahrungen mit dem Phytotherapeutikum Umckaloabo®. Therapiewoche Pädiatrie 7, 523–525.

Helmstädter, A., 1996. Umckaloabo—late vindication of a secret remedy. Pharmaceutical Historian 26, 2–4.

Hunter, C.A., Sanders, J.K.M., 1990. The nature of π–π interactions. Journal of the American Chemical Society 112, 5525–5534.

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

Kayser, O., Latté, K., Kolodziej, H., Hammerschmidt, F.-J., 1998. Composition of the essential oils of *Pelargonium sidoides* DC and *Pelargonium reniforme* CURT. Flavour and Fragrance Journal 13, 209–212.

König, I., 1995. Naturnahe Atemwegstherapie. Von der Umckaloabo-Droge zur Therapie von Atemwegsinfekten. Therapiewoche 45, 1123–1124.

Latté, K.P., Kolodziej, H., 2000. Pelargoniins, new ellagitannins from *Pelargonium reniforme* CURT. 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. Zeitschrift für Naturforschung 55c, 528–533.

Leitao, S.G., Monache, F.D., 1998. 2″-*O*-Caffeoylorientin from *Vitex polygama*. Phytochemistry 49, 2167–2169.

Maatooq, G.T., El-Sharkawy, S.H., Afifi, M.S., Rosazza, J.P.N., 1997. *C*-*p*-Hydroxybenzoylglycoflavones from *Citrullus colocynthis*. Phytochemistry 44, 187–190.

Markham, K.R., Mues, R., Stoll, M., Zinsmeister, H.D., 1987. NMR spectra of flavone di-*C*-glycosides from *Apometzgeria pubescens* and the detection of rotational isomerism in 8-*C*-hexosylflavones. Zeitschrift für Naturforschung 42c, 1039–1042.

Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T., Still, W.C., 1990. Macromodel-an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. Journal of Computational Chemistry 11, 440–467.

Rabe, C., Steenkamp, J.A., Joubert, E., Burger, J.F.W., Ferreira, D., 1994. Phenolic metabolites from rooibos tea (*Aspalathus linearis*). Phytochemistry 35, 1559–1565.

Shen, C., Chang, Y., Ho, L., 1993. Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids. Phytochemistry 34, 1976–1981.

Sood, A.R., Boutard, B., Chadenson, M., Chopin, J., Lebreton, P., 1976. A new flavone *C*-glycoside from *Trigonella foenum-graecum*. Phytochemistry 15, 351–352.

Steynberg, J.P., Brandt, E.V., Ferreira, D., 1991. Conformational analysis of oligomeric flavanoids. Part 1. 4-Arylflavan-3-ols. Journal of the Chemical Society, Perkin Transactions 2, 1569–1573.

Van der Walt, J.J.A., Vorster, P.J., 1983. Phytogeography of *Pelargonium*. Bothalia 14, 517–523.

Watt, C., Breyer-Brandwijk, M. G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edinburgh, London, pp. 449–455.

Williams, C.A., Newman, M., Gibby, M., 2000. The application of leaf phenolic evidence for systematic studies within the genus *Pelargonium* (Geraniaceae). Biochemical Systematics and Ecology 28, 119–132.