Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 905–911
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Caffeoyl sugar esters and an ellagitannin from *Rubus sanctus*

Sahar A.M. Hussein[a], Nahla A. Ayoub[b], Mahmoud A.M. Nawwar[a,*]

[a]*National Research Center, Dokki, Cairo, Egypt*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt*

Received 30 January 2003; received in revised form 7 May 2003

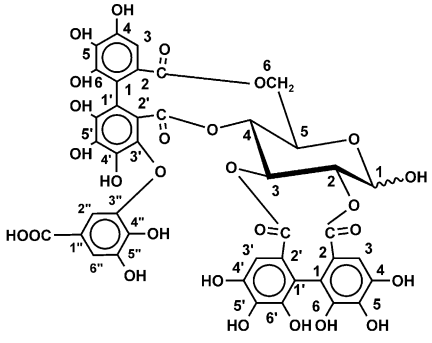
Abstract

The new natural caffeoyl esters, 3,6-di-*O*-caffeoyl-(α/β)-glucose and 1-*O*-caffeoyl-β-xylose, together with the hitherto unknown natural tannin, 2,3-O-hexahydroxydiphenoyl-4,6-*O*-sanguisorboyl-(α/β)-glucose, have been isolated from the aqueous alcohol aerial part extract of *Rubus sanctus*. Establishment of all structures was based on the chemical and spectral evidence, including ESI–MS and 2D NMR.

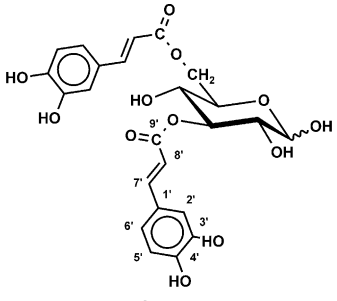
Keywords: *Rubus sanctus*; Rosaceae; Caffeoyl esters; 3,6-di-*O*-caffeoyl-(α/β)-glucose; 1-*O*-caffeoyl-β-xylose; Tannin; 2,3-*O*-hexahydroxydiphenoyl-4,6-*O*-sanguisorboyl-(α/β)-glucose; ESI-MS; 2D NMR

1. Introduction

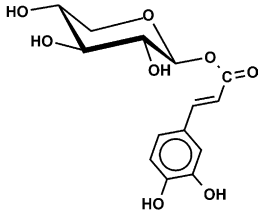
Rubus species (Rosaceae) are known to provide extracts which have been used in traditional medicine as antimicrobial (Richard et al., 1994; Rauha et al., 2000), anticonvulsant, muscle relaxant (Nogueira and Vassilieff, 2000) and radical scavenging (Constantino et al., 1992) agents. They are characterized by their capability of synthesizing and accumulating ellagitannins containing a sanguisorboyl group (Tanaka et al., 1993). They have also been found to metabolise several phenolic carboxylic acids, such as ellagic acid, and phenyl propenoids, particularly caffeic acid (Häkkinen et al., 1999). In a continuation of our chemical studies on phenolics in Rosaceous plants, we are investigating, in the present communication, *Rubus sanctus* Schreb., which in contrast to several other *Rubus* species, has not yet been examined for its constitutive phenolics. As a result, we were able to isolate and identify the new natural products, 3,6-di-O-caffeoyl-(α/β)-glucose (**1**) and 1-O-caffeoyl-β-xylose (**2**), together with the hitherto unknown natural tannin, 2,3-*O*-hexahydroxydiphenoyl-4,6-*O*-sanguisorboyl-(α/β)-glucose (**5**), in addition to the known tannins, 2,3-hexahydroxydiphenoyl-(α/β)-glucose (**3**) and bis-2,3,4,6-hexahydroxydiphenoyl-(α/β)-glucose (**4**) from the aqueous alcohol extract of the aerial parts of the plant. It should be mentioned, however, that *Rubus sanctus* Schreb. is the only *Rubus* species that grows. wild in Egypt. The plant, known in Egypt as Olleiqa, is a 1–2 m shrub of a prostrate or arching stem, ternate or palmate leaves with five leaflets, numerous flowers in lax panicles and which produces fruits with several black, glabrous and juicy drupelets (Bolous, 1999).



Compound **(5)**
2,3-O-hexahydroxydiphenoyl-4,6-O-sanguisorboyl glucose



Compound **(1)**
3,6-di-O-caffeoyl glucose



Compound **(2)**
1-O-caffeoyl xylose

* Corresponding author. Fax: +20-2-337093.
E-mail address: nawwar@worldnet.com.eg (M.A.M. Nawwar).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00331-5

2. Results and discussion

The aqueous ethanolic (75%) extract, obtained at room temperature, from a homogenate of the ground dried aerial parts of *R. sanctus*, was shown by preliminary two-dimensional paper chromatographic screening to contain a complicated phenolic mixture (positive response towards $FeCl_3$ spray reagent) from which five compounds (**1–5**) were isolated and purified through a series of fractionations on Sephadex LH-20 and polyamide columns.

Compounds (**3** and **4**) were found to be known and gave chromatographic, UV absorption, hydrolytic, ESI–MS, ^1H and ^{13}C NMR data identical with those reported for 2,3-*O*-hexahydroxydiphenoyl-(α/β)-glucopyranose (Tanaka et al., 1991) and pedunculagin (Tanaka et al., 1993).

The new phenolic (**1**) was obtained as an amorphous off-white powder which possesses caffeoyl ester-like characteristics (fluorescent blue spot on paper chromatograms under UV light, changing to a bright canary yellow colour on fuming with ammonia, an intense green colour with $FeCl_3$ and UV spectral maxima in MeOH; Table 1), (Harborne, 1973). Negative ESI-MS spectrum of (**1**) exhibited a molecular ion $[M\text{-}H]^-$ at $m/z = 503$ and fragment ions, $[M\text{-caffeoyl}]^-$ at $m/z = 341$ and $[M\text{-}2\text{ caffeoyl} + H]^-$ at $m/z = 179$, corresponding to a molecular mass of 504 and a molecular formula of $C_{24}H_{24}O_{12}$ (elemental analysis: found: C = 56.82%, H = 4.43%; calculated: C = 57.14%, H = 4.76%). This view was supported by complete hydrolysis with 1 N aqueous NaOH (3 h at 100 °C) of (**1**), which yielded caffeic acid (comparative paper chromatography, UV, ^1H and ^{13}C NMR spectral analysis) together with glucose (Comparative paper chromatography). On partial acid hydrolysis, by 0.5 N aqueous NaOH (3 h at 100 °C) compound (**1**) gave, besides glucose and caffeic acid (comparative paper chromatography), an intermediate (**1a**) which was purified from an ethyl acetate extract of the acidified hydrolysate, by preparative paper chromatography. (**1a**) was shown to possess a molecular mass of 342 (negative ESI–MS molecular ion $[M\text{-}H]^-$ at m/z 341and UV spectral maxima in MeOH; Table 1) thus suggesting its structure to be a mono-*O*-caffeoyl glucose. This suggestion was proved through ^1H NMR analysis of (**1a**). The spectrum (DMSO-d_6, room temperature) showed the presence of a free anomeric glucose hydroxyl group. This unequivocally followed from the two doublets at δ 5.16 ($J = 3$ Hz) and at δ 4.52 ppm ($J = 7.5$ Hz), assignable to the resonances of the α- and β- anomeric protons, respectively. The spectrum also showed a pair of doublets at δ ppm 4.32 and 4.42 (both with $J = 12.5$ Hz) as well as a pair of double doublets at δ ppm 4.25 and 4.28 (both with $J = 4$ Hz and 12.5 Hz), attributable to the two H-6 methylenic glucose protons in both anomers of (**1a**). These lowfield methylenic proton resonances [in comparison with the corresponding resonances in the spectrum of free α/β-glucose (Nawwar et al., 1984)] confirmed that the caffeoyl moiety in (**1a**) is located at C-6 of the glucose core. As expected, each of the resonances of the caffeoyl moiety itself revealed its presence, in duplicate in the recorded spectrum (see Section 3). Consequently, intermediate (**1a**) is 6-mono-*O*-caffeoyl-(α/β)-glucose (Shimomur et al., 1988). To find out the site of attachment of the second caffeoyl moiety in the molecule of the parent compound (**1**), NMR spectral analysis was then performed. The spectrum (DMSO-d_6, room temperature) revealed two different patterns of proton resonances belonging to an α/β anomeric mixture of disubstituted glucose, whereby a pair of doublets, centred at δ ppm 5.15 $J = 3.5$ Hz) and at δ 4.65 ($J = 8$ Hz) were recognized, thus indicating the presence of a free anomeric hydroxyl group.

The spectrum also showed two downfield sugar resonances at δ 5.20 (*t*, $J = 8$ Hz) and at δ 4.95 (*t*, $J = 8$ Hz) assignable to the H-3 glucose protons in both α- and β-anomers, respectively. This assignment was confirmed by a COSY experiment. Acylation at the 6-position of glucose in (**1**) was further evidenced by the lowfield methylenic proton resonances located at δ 4.38 (*d*, $J = 12.5$ Hz), 4.34 (*d*, $J = 12.5$ Hz), 4.20 (*dd*, $J = 12.5$ and 4.5 Hz) and 4.14 (*dd*, $J = 12.5$ and 4.5 Hz). In addition,

Table 1
Chromatographic and UV spectral data of compounds (**1–5**)

Compound	Chromatographic properties			UV Spectral data λ_{maz} nm, in MeOH
	R_f- (× 100)			
	H_2O	HOAC	BAW	
3,6-di-*O*-caffeoyl glucose (**1**)	75	78	34	$250_{shoulder}$, 295, 334
6-mono-*O*-caffeoyl glucose (**1a**)	62	68	45	$242_{shoulder}$, 302, 330
Caffeic acid	25	56	81	245, 298, 325
1-mono-*O*-caffeoyl xylose (**2**)	52	63	55	$242_{shoulder}$, 300, 333
2,3-*O*-hexahydroxydiphenoyl- glucose (**3**)	66	76	29	$262_{shoulder}$
Bis-2,3;4,6-*O*-hexahydroxy-Diphenoyl-(α/β)-glucose, pedunculagin (**4**)	58	70	35	$260_{shoulder}$
2,3-*O*-hexahydroxydiphenoyl- 4,6-O-sanguisorboyl-(α/β)-glucose (**5**)	59	72	37	$260_{shoulder}$

the values of the above given coupling constants indicate that the glucose core of (**1**) is adopting a $^4C_1$-conformation. The spectrum also, revealed the presence, in duplicates, of two *trans* olefinic systems together with aromatic protons of two ABC systems with chemical shifts, multiplicities and coupling constants (see Section 3) typical for caffeoyl moieties. Consequently, compound (**1**) is 3,6-di-*O*-caffeoyl-(α/β)-glucose. The ^{13}C NMR spectrum of (**1**) showed, as expected, a duplicated resonance pattern of glucose carbon resonances. Assignments in this spectrum were made by comparison with the ^{13}C NMR data, reported for similar acylated glucoses (Haddock et al., 1982) and by consideration of the known α- and β-effect caused by esterifying the sugar hydroxyl groups (Kalinowski et al., 1984). The α- and β- anomeric carbon resonances were readily identified from their characteristic chemical shift values [δ 92.9 (C-α) and 97.5 (C-β)]. Acylation at the glucose C-3 position followed from the lowfield shift (α-effect) of its carbon resonances, in both anomers (Δ δ ppm 3.8 for C-3-α and 1.5 for C-3-β) and also from the accompanying upfield shift (β-effect) of the vicinal carbon resonances (Δ δ ppm 0.7 for C-2-α and 1.2 for C-2-β), all in comparison with the corresponding resonances in the spectrum of free (α/β)-glucopyranose (Nawwar et al., 1984). Attachment of the second caffeoyl moiety of (**1**) at C-6 of the glucose core was evidenced by the downfield shift (α-effect) of its carbon resonances, in both anomers to δ ppm 64.09 and 64.5 and also, by the upfield shift (β-effect) of the resonances of the vicinal C-5 carbons to δ ppm 69.8 and 74.1, all in comparison with the chemical shifts of the corresponding resonances in the spectrum of free (α/β)-glucopyranose (Nawwar et al., 1984). Other resonances in this spectrum exhibited chemical shift values which were in accordance with the achieved structure of (**1**). Furthermore, the measured chemical shifts of the glucose carbon resonances proved that this moiety existed in the pyranose form, thus finally confirming the structure of (**1**) to be 3,6-di-*O*-caffeoyl-(α-β)-$^4C_1$-glucopyranose, which represents, to the best of our knowledge, a new natural product.

Compound (**2**) was separated as an amorphous white powder. On paper chromatograms (R_f-values; Table 1) it exhibited the characteristic UV fluorescences of caffeoyl esters. Its UV absorption spectrum (Table 1) was also typical for these esters. It was analysed for $C_{14}H_{18}O_9$ through negative ESI–MS (molecular ion $[M\text{-}H]^-$ at $m/z = 329$) and elemental analysis (found: C 51.88%, H 4.36%; calculated: C 50.90%, H 5.45%) as well. On normal alkaline hydrolysis (1 N aqueous NaOH, 100 °C, 3 h) compound (**2**) yielded caffeic acid [comparative paper chromatography, UV maxima (Table 1), EI–MS spectrum] and xylose (comparative paper chromatography). These analytical data showed that (**2**) must be a mono-caffeoyl-*O*-xylose. Establishment of the final structure was then achieved through NMR spectroscopy. The ^1H spectrum (DMSO-d_6, room temperature) revealed two distinct patterns of proton resonances, the first was typical for esterified caffeic acid and contained well-separated resonances of this moiety [(δ ppm 6.25 (*d*, *J* = 16 Hz, H-8), 6.85 (*d*, *J* = 8 Hz, H-5), 7.0 (*dd*, *J* = 8 Hz and 2.5 Hz, H-6), 7.04 (*d*, *J* = 2.5 Hz, H-2), 7.6 (*d*, *J* = 16 Hz, H-7)]. The second pattern was characteristic for sugar protons and contained an anomeric resonance at δ ppm 5.65 (*d*, *J* = 7 Hz). This chemical shift value indicated esterification of the anomeric hydroxyl group, while the measured coupling constant proved the β-configuration of the xylose moiety which must, therefore adopt a $^4C_1$ conformation. Other xylose proton resonances possessed a chemical shift between δ ppm 3.3 and 3.8 (see Section 3), thus proving the structure of the compound (**2**) as 1-*O*-caffeoyl-β-$^4C_1$-xlose. From the ^{13}C spectrum of (**2**), the presence of a β-xylose moiety acylated at its anomeric carbon followed from the chemical shift value of the resonance at δ ppm 93.4, attributable to the anomeric carbon, a value which is a relatively upfield shifted when compared with the value of the corresponding resonance in the spectrum of free β-xylose (Kalinowski et al., 1984). That the carboxyl group of the caffeoyl moiety is esterified followed from the upfield shift of the carbonyl carbon resonance (see Section 3) in comparison with the the corresponding resonance in the spectrum of free caffeic acid (El-Mousallamy et al., 2000). The measured chemical shifts of the xylose carbon resonances proved that this moiety existed in the pyranose form. Other resonances in this spectrum, possessed chemical shifts which were in full agreement with the determined structure of (**2**) as 1-*O*-caffeoyl-β-$^4C_1$-xylopyranoside, a new caffeoyl sugar ester which has not been reported before as occurring in nature.

Compound (**5**), the new tannin (**5**), was isolated as an off-white, amorphous powder of $[\alpha]_{D+}$17.5° (acetone; *c* 1.7). It showed chromatographic properties (dark blue spot on paper chromatograms under short UV light, R_f-values, Table 1), colouration with $FeCl_3$ (dark blue) and $NaNO_2$-HOAc (orange-dark greenish blue) reagents and UV spectral data (Table 1) characterisitic for ellagitannins (Gupta et al., 1982). Acid hydrolysis (5% aqueous H_2SO_4, 100 °C, 6 h) of compound (**5**), followed by extraction of the hydrolysate by ethyl acetate and preparative paper chromatography of the obtained extract yielded ellagic acid and sanguisorbic acid which were fully characterized through UV, negative ESI–MS and ^1H NMR spectral analysis. The sugar released during hydrolysis was proved to be glucose (comparative paper chromatography). Compound (**5**) exhibited an $[M + Na]^+$ ion at $m/z = 975$, in its positive ESI–MS spectrum and an $[M\text{-}H]^-$ ion at $m/z = 951$, in its negative ESI–MS spectrum, corresponding to a molecular mass of 952 and a molecular formula of $C_{41}H_{28}O_{27}$ (found, C 52.05%, H 2.70%; calculated, C 51.80%, H 2.94%).

The latter spectrum also showed fragment ions at $m/z = 649$ [M-hexahydroxydiphenoyl]$^-$, 469 [mass of sanguisorbic acid-H]$^-$ and at 301 [mass of ellagic acid-H]$^-$, thus proving, together with the above given data, that compound (**5**) is a hexahydroxydiphenoyl–sanguisorboyl glucose. In order to unravel ambiguity about the site of attachment of both the diphenoyl and the sanguisorboyl moieties to the glucose core in the molecule of (**5**), NMR analysis was undertaken. The ^{1}H spectrum (DMSO-d_6, room temperature) revealed proton resonances, almost in duplicates, of hexahydroxydiphenoyl moiety [6.33, 6.39 (1H in total, each s, H-3′), 6.50, 6.55 (1H in total, each *s*, H-3)], a sanguisorboyl moiety [6.61, 6.63 (1H in total, each *s*, H-3), 7.03, 7.04 (1H in total, each *d*, $J = 2$Hz, H-6″), 7.08, 7.09 (1H in total, each *d*, $J = 2$Hz, H-2″)] and of glucopyranose core [α-anomer: 5.25 (*d*, $J = 2$ Hz, H-1), 5.05 (*dd*, $J = 8$Hz and $J = 2$Hz, H-2), 4.7–4.8 (*m*, H-3 and H-4), 4.18 (*m*, H-5), 5.52 (*dd*, $J = 13$ Hz, $J = 6$ Hz, H-6), 3.74 (*d*, $J = 13$ Hz, H-6); β-anomer: 4.83 (*d*, $J = 8$ Hz, H-1), 4.66 (*t*, $J = 8$ Hz, H-2), 4.7-4.8 (*m*, H-3 and H-4), 4.18 (*m*, H-5), 5.50 (*dd*, $J = 13$ and $J = 6.5$ Hz, H-6), 3.7 (*d*, $J = 13$ Hz, H-6)]. Examination of the ^{1}H-^{1}H COSY spectrum of (**5**) indicated that the resonance at δ 5.25 due to H-1 of the α-glucose moiety was correlated with the H-2 resonance at δ 5.05, which was further correlated with the multiplet resonance of H-3 and H-4 at δ 4.7–4.8 ppm. In this spectrum, off-diagonal cross peaks proving the sequential correlation of the resonances of H-4, H-5 and H-6 were also determined. Assignments of the proton resonances of the β-glucose moiety were confirmed in the same way. Duplication of almost all of these resonances indicated that (**5**) formed an anomeric mixture due to the presence of a free anomeric hydroxyl group. The coupling constants of the glucose proton are characterisitc of those of a glucopyranose core adopting the $^4C_1$ conformation. The large upfield shift, given above, for the H-3 and H-5 glucose resonances, together with the unusual lowfield shift of one of the H-6 resonances of this moiety, as compared with those of pedunculagin [5.22, 5.27 (H-3), 4.48, 4.27 (H-5) and 5.06, 5.09], are typical for 4,6-sanguisorboyl glucoses (Tanaka et al., 1993). The remaining hexahydroxydiphenoyl moiety is hence attached at the C-2 and C-3 glucose positions, thus proving the structure of compound (**5**) to be 2,3-*O*-hexahydroxydipheoyl-4,6-*O*-sanguisorboyl-(α/β)-$^4C_1$-glucopyranose. The ^{13}C spectrum of (**5**) exhibited, almost in duplicate (Table 2), the resonances of a hexahydroxydiphenoyl, and a sanguisorboyl moiety, together with 12 resonances due to oxygen-bearing aliphatic carbons, including two anomeric signals with chemical shift values [90.5 (C-1α), 94.5 (C-1β)], which were closely related to those of the anomeric carbons in pedunculagin [90.3 (C-1α), 93.8 (C-1β)]. The assignments of the carbon resonances, in this spectrum (see Section 3, Table 2), were greatly aided by comparison with the ^{13}C NMR data reported for analogous compounds, bearing 4,6-*O*-sanguisorboyl glucose moiety (Tanaka et al., 1993) and was confirmed by 2D-HETCOR experiment. These data finally confirmed the structure of compound (**5**) to be 2,3-O-hexahydroxydipheoyl-4,6-O-sanguisorboyl-

Table 2
^{13}C chemical shifts (δ ppm) of compounds (**4** & **5**)

Carbon	Compound (**4**)	Compound (**5**)
α-glucose		
1	90.30	90.50
2	74.29	74.50
3	75.82	76.52
4	68.70	69.37
5	66.20	66.69
6	62.50	62.80
β-glucose		
1	93.80	94.50
2	77.25	77.25
3	75.63	74.45
4	71.63	69.73
5	70.80	72.00
6	61.40	62.80
HHDP in α-& β-anomers		
1 & 1′	113.18, 113.30	114.65, 114.74, 114.87, 115.12
	113.84, 113.95, 115.09, 115.54,	124.39, 124.58, 124.98, 125.05
2 & 2′	123.61, 124.45, 124.52	105.3, 105.8, 106.3
3 & 3′	105.4, 105.6, 105.8	144.50, 144.56, 144.64, 144.96, 145.10, 145.26, 145.33
4, 4′,6 & 6′	144.36, 144.39, 144.48, 144.53, 144.66, 144.70, 144.81	
5 & 5′	134.70, 134.77, 135.03,135.41	134.40, 134.61, 135.28, 135.43
C=O	167.13, 167.19,167.63, 167.73, 168.24, 168.65	167.98, 168.00, 168.23, 168.32,
Sanguisorbyl in α-& β-anomer		
1		114.18, 114.30
1′		109.90, 110.21
2		125.22, 125.46
2′		116.20, 116.22
3		106.39
3′		137.00, 137.03
4,6		145.34, 145.43, 145.64
4′		140.13, 140.46
5		136.85, 136.87
5′		138.27, 138.49
6′		116.20, 116.21
1″		142.63, 142.70
2″		111.14, 111.20
3″		147.80, 147.84
4″		138.22, 138.27
5″		146.19, 146.41
6″		109.90, 110.21
C=O		165.76, 167.80, 167.94, 167.96, 168.70, 168.82

(α/β)-$^4C_1$-glucopyranose, which has not been reported before as a natural product.

3. Experimental

^{1}H NMR spectra were measured at 300 MHz, on a Jeol-YH-300 NMR spectrometer. ^{1}H chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 4000 Hz for ^{1}H and 19 000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. ESI–MS spectra were measured on SSQ Finnigan MAT 4600 quadrupole mass spectrometer (Institut für Chemie, Humboldt Universität, Berlin). Paper chromatographic analysis was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) 6% HOAc; (3) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer); (4) C_6H_6-*n*–BuOH–H_2O–pyridine (1:5:3:3, upper layer). Solvents 1 and 3 were used for preparative paper chromatography on Whatman No. 3MM. Solvents 3 and 4 were used for sugar analysis.

3.1. Plant material

Fresh shrubs of *Rubus sanctus* Schreb., were collected from South Sinai, near Saint Katherine city, Egypt, during November 2000 and authenticated by Dr M. El-Gibali, National Research Centre (NRC), cairo, Egypt. A voucher specimen is deposited at the NRC.

3.2. Isolation and identification

The ground dried aerial parts of *R. sanctus* were homogenized in EtOH–H_2O (1:3) mixture. (3 Kg, three extractions each with 5 l.). The concentrated filtrate of the homoginate was applied to a Sephadex LH-20 column (125×5 cm inte) and eluted with water followed by H_2O–MeOH mixtures of decreasing polarities to yield two phenolic fractions (paper chromatography), eluted by H_2O–MeOH (90:10) and (10:90). Compounds (**1–3**) were isolated, from the 10% fraction by applying repeated Sephadex LH-20 column fractionation, using water for elution, which led to the successive desorption of these compounds. Preparative paper chromatography of the crude materials of (**1–3**), using BAW as solvent afforded pure samples [(**1**, 94 mg); (**2**, 101 mg) and (**3**, 141 mg)] of each. Compounds (**4** and **5**) were isolated, from the 90% fraction by applying polyamide 6 S (Riedel-De Häen AG, Seelze Hanover, Germany) column fractionation and elution with H_2O–EtOH (30:70) mixture, followed by repeated Sephadex LH-20 column fractionation of the desorbed crude materials of (**4** and **5**), using MeOH for elution, which led to the isolation of individual pure samples of [(**4**, 134 mg) and (**5**, 110 mg)].

3.3. 3,6-di-O-caffeoyl-(α/β)-glucose (1)

R_f-values: Table 1. UV λ_{max}^{MeOH} nm: ESI–MS of (**1**): negative molecular ion [M-H]$^-$: $m/z = 503$ and fragment ions: $m/z = 341$ [M-caffeoyl]$^-$, 179 [m-2 caffeoyl]$^-$. Complete alkaline hydrolysis (28 mg of **1** refluxed with 10 ml aqueous 1 N NaOH, at 100 °C, for 3 h). Acidification of the hydrolysate with aqueous 2 N HCl, followed by extraction by ethyl acetate, washing of the organic layer with water, filtration through anhydrous Na_2SO_4 and comparative paper chromatography proved the presence of caffeic acid: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ^{1}H NMR: δ ppm: 6.2 (*d*, *J* = 16 Hz, H-8), 6.76 (*d*, *J* = 7.5 Hz, H-5), 6.88 (*dd*, *J* = 7.5 and 2.5 Hz, H-6), 6.98 (*d*, *J* = 2.5 Hz, H-2), 7.48 (*d*, *J* = 16 Hz, H-7); ^{13}C NMR: δ ppm: 126.1 (C-1), 115.2 (C-2), 144.9 (C-3), 148.4 (C-4), 116.2 (C-5), 121.5 (C-6), 146.2 (C-7), 115.2 (C-8), 168.0 (C-9); The remaining acidified hydrolysate was set acid free through extraction with *N*,*N*-dimethyloctyl amine in $CHCl_3$ then concentrated in vacuo and examined by comparative paper chromatography to prove the presence of glucose. Partial acid hydrolysis (48 mg of **1**, 10 ml aqueous 0.5 N NaOH, 100 °C, 3 h) yielded glucose, caffeic acid and $\mathbf{1_a}$: *Rf*-values (Table 1); UV λ_{max}^{MeOH} nm: Table 1; ESI–MS negative molecular ion: $m/z = 341$ [M-H]$^-$; ^{1}H NMR : Caffeoyl moiety: δ ppm: 6.25, 6.26 (1 H in total, each as *d*, *J* = 16 Hz, H-8′),6.78, 6.79 (1H in total, each as *d*, *J* = 7.5 Hz, H-5′), 6.94 (1 H in total, *m*, H-6′), 7.04, 7.05 (1H in total, each as *d*, *J* = 2.5 Hz, H-2′), 7.56, 7.57 (1 H in total, d, *J* = 16 Hz, H-7′); α-glucose moiety: δ ppm: 5.16 (*d*, *J* = 3 Hz, H-1), 4.42 (*d*, *J* = 12.5 Hz, H-6_a), 4.28 (*dd*, *J* = 12.5 Hz and 4 Hz, H-6_b), 3.25–3.85 (*m*, overlapping other sugar and hydroxyl protons); β-glucose moiety: δ ppm: 4.52 (*d*, *J* = 7.5 Hz, H-1), 4.32 (*d*, *J* = 12.5 Hz, H-6_a), 4.25 (*dd*, *J* = 12.5 Hz and 4 Hz, H-6_b), 3.25–3.85 (*m*, overlapping other sugar and hydroxyl protons). ^{1}H NMR of (**1**): δ ppm: Caffeoyl moiety: δ ppm: 6.23, 6.25 (1 H in total, each as *d*, *J* = 16 Hz, H-8′), 6.75, 6.77 (1 H in total, each as *d*, *J* = 7.5 Hz, H-5′), 7.0 (1 H in total, *m*, H-6′), 7.04, 7.05 (1 H in total, each as *d*, *J* = 2.5 Hz, H-2′), 7.46, 7.50 (1 H in total, *d*, *J* = 16 Hz, H-7′); α-glucose moiety: δ ppm: 5.15 (*d*, *J* = 3 Hz, H-1), 5.20 (*t*, H = 8 Hz, H-3), 4.38 (*d*, *J* = 12.5 Hz, H-6_a), 4.20 (*dd*, *J* = 12.5 4 Hz, H-6_b), 3.25–3.85 (*m*, overlapping other sugar and hydroxyl protons); β-glucose moiety: δ ppm: 4.65 (*d*, *J* = 8 Hz, H-1),4.95 (*t*, *J* = 8 Hz, H-3), 4.34 (*d*, *J* = 12.5 Hz, H-6_a), 4.14 (*dd*, *J* = 12.5 Hz and 4 Hz, H-6_b), 3,25-3.85 (*m*, overlapping other sugar and hydroxyl protons). ^{13}C chemical shifts: (Table 2).

3.4. 1-O-caffeoyl-β-xylose (2)

R_f-values: Table 1. UV λ_{max}^{MeOH} nm: ESI-MS of (**1**): negative molecular ion [M-H]$^-$: $m/z = 329$. Complete

alkaline hydrolysis (21 mg of **2** refluxed with 10 ml aqueous 1 N NaOH, at 100 °C, for 3 h). Acidification of the hydrolysate with aqueous 2 N HCl, followed by extraction by ethyl acetate, washing of the organic layer with water, filtration through anhydrous Na_2SO_4 and comparative paper chromatography proved the presence of caffeic acid: R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ESI:=MS negative ion $[M-z]^-$ at $m/z=179$; The remaining acidified hydrolysate was set acid free through extraction with *N*,*N*-dimethyloctyl amine in $CHCl_3$ then concentrated in vacuo and examined by comparative paper chromatography to prove the presence of xylose. ^{1}H NMR of (**2**): δ ppm: Caffeoyl moiety: δ ppm: 6.25 (1 H, d, $J=16$ Hz, H-7′), 6.85 (1 H, *d*, $J=8$ Hz, H-5′), 7.0 (1 H, *m*, H-6′), 7.04 (1 H, *d*, $J=2.5$ Hz, H-2′), 7.60 (1 H, *d*, $J=16$ Hz, H-7′); β-xylose moiety: δ ppm: 5.65 (*d*, $J=7$ Hz, H-1), 3,3-3.8 (*m*, overlapping other sugar and hydroxyl protons). ^{13}C chemical shifts: (Table 2).

3.5. 2,3-O-hexahydroxydiphenoyl-(α/β)-glucose (3)

R_f-values: Table 1. UV λ_{max}^{MeOH} nm: Table 1. ESI-MS negative molecular ion $[M-H]^-$: $m/z=481$. ^{1}H NMR: δ ppm: Hexahydroxydipheoyl moiety: δ ppm: 6.3, 6.31 (1 H in total, *s*, H-3′), 6.42, 6.43 (1 H in total, *s*, H-3″); (α/β)-glucose moieties: δ ppm: 5.25 (1 H, *d*, $J=2.5$ Hz, H-1α), 4.88 (1 H, *d*, $J=8$ Hz, H-1β), 5.13 (1 H, *t*, $J=8$ Hz, H-3α), 5.05 (1 H, *t*, $J=8$ Hz, H-3β), 4.81 (1 H, *dd*, $J=8$ Hz and 2.5 Hz, H-2α), 4.5 (1H, t, $J=8$ Hz, H-2β), 3.4–3.7 (*m*, overlapping other sugar and hydroxyl protons).

3.6. Bis-2,3;4,6-O-hexahydroxydiphenoyl-(α/β)-glucose, pedunculagin (4)

R_f-values: Table 1. UV λ_{max}^{MeOH} nm: Table 1. ESI–MS negative molecular ion $[M-H]^-$: $m/z=783$. ^{1}H NMR: δ ppm: Hexahydroxydipheoyl moieties: δ ppm: 6.3, 6.34, 6.38, 6.39 (2 H in total, each *s*, H-3′, in both moieties), 6.4, 6.42, 6.425, 6.43 (2 H in total, each *s*, H-3″, in both moieties); (α/β)-glucose moieties: δ ppm: 5.35 (*d*, $J=2.5$ Hz, H-1α), 4.89 (*d*, $J=8$ Hz, H-1β), 5.27 (*t*, $J=8$ Hz, H-3α), 5.22 (*t*, $J=8$ Hz, H-3β), 5.05 (*dd*, $J=12.5$ Hz and $J=4$ Hz, H-6α), 5.09 (*dd*, $J=12.5$ 4 Hz, H-6β), 4.83 (*dd*, $J=8$ 2.5 Hz, H-2α), 4.62 (*t*, $J=8$ Hz, H-2β), 4.9 (*t*, $J=8$ Hz, H-4α), 4.82 (*t*, $J=8$ Hz, H-4β), 4.48 (*m*, H-5α), 4.27 (*m*, H-5β), 3.82(*d*, $J=12.5$ Hz, H-6α), 3.78 (*d*, $J=12.5$, H-6β). ^{13}C NMR: Table 2.

3.7. 2,3-O-hexahydroxydiphenoyl-4,6-O-sanguisorboyl-(α/β)-glucose (5)

R_f-values: Table 1. UV λ_{max}^{MeOH} nm: Table 1. ESI–MS: negative molecular ion $[M-H]^-$: $m/z=951$. Acid hydrolysis: 33 mg of **5** + 10 ml aqueous 5% H_2SO_4, 100 °C, 6 h, yielded glucose, ellagic acid and sanguisorbic acid; Ellagic acid: R_f-values : Table ; UV λ_{max}^{MeOH} nm: Table 1; ESI–MS: negative ion $[M-H]^-$: $m/z=301$; ^{1}H NMR ^{1}H: 7.5 (*s*, H-3 and H-3′); Sanguisorbic acid: R_f-values : Table ; UV λ_{max}^{MeOH} nm: Table 1; ESI–MS: negative ion $[M-H]^-$: $m/z=469$; ^{1}H NMR: δ ppm: 6.45 (*d*, $J=2$ Hz, H-2), 7.02 (*d*, H = 2 Hz, H-6), 7.5 (*s*, H-3′). ^{1}H NMR of **5**: δ ppm: Hexahydroxydipheoyl moieties: δ ppm: 6.33, 6.39, (1 H in total, each *s*, H-3′), 6.5, 6.55 (1 H in total, each *s*, H-3); sanguisorboyl moiety: δ ppm: 6.61, 6.63 (1 H in total, each *s*, H-3′), 7.03, 7.04 (1 H in total, *d*, J-2 Hz, H-6), 7.06, 7.09 (1 H in total, *d*, $J=2$ Hz, H-2); (α/β)-glucose moieties: δ ppm: 5.25 (*d*, $J=2.5$ Hz, H-1α), 4.83 (*d*, $J=8$ Hz, H-1β), 5.05 (*dd*, $J=8$ Hz and 2 Hz, H-2α), 4.66 (*t*, $J=8$ Hz, H-2β), 4.7–4.8 (*m*, H-3α, H-3β, H-4α and H-4β), 4.18 (*m*, H-5α), 4.08 (*m*, H-5β), 5.52 (*dd*, $J=13$ 6 Hz, H-6α), 5.50 (*dd*, $J=13$ Hz and 6 Hz, H-6β), 3.74 (*d*, $J=12.5$ Hz, H-6α), 3.70 (*d*, $J=12.5$, H-6β). ^{13}C NMR: Table 2.

Acknowledgements

The authors wish to express their deep gratitude to Professor Dr. M. Linscheid, Institut für Chemie, Humboldt-University Berlin for the ESI-MS measurements.

References

Bolous, L., 1999. Flora of Egypt, Vol. 1. Al Hadara Publishing, Cairo, Egypt.

Constantino, L., Albasini, A., Rastelli, G., Benvenuti, S., 1992. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthin oxidase. Planta Medica 58, 343.

El-Mousallamy, A., Hawas, U., Hussein, S., 2000. Teucrol, a decarboxyrosnarenic acid and its 4′-*O*-triglycosides, teucroside from *Teucrium pilosum*. Phytochemistry 55, 927.

Gupta, R.K., Al-Shafi, S.M., Layden, K., Haslam, E., 1982. The metabolism of gallic acid and hexahydroxydiphenic acid in plants. Part 2, Esters of 9s)-hexahydroxydiphenic acid with O-glucopyranose. J. Chem. Soc., Perkin Trans. 1 2525.

Haddock, A., Gupta, R., Al-Shafi, S., Layden, K., Haslam, E., 1982. The metabolism of gallic acid and hexahydrooxydiphenic acid in plants. Part 1: Introduction. Naturally occuring galloyl esters. J. Chem. Soc., Perkins Trans. 1 2525.

Harborne, H.B., 1973. Phytochemical Methods. Chapman & Hall, London.

Häkkinen, S., Neinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., Törrönen, R., 1999. Screening of selected flavonoids and phenol acids in 19 berries. Food Research International 32, 345.

Kalinowski, H.O., Berger, S., Braun, S., 1984. ^{13}C NMR Spektroskopie. George Thieme, Stuttgart.

Nawwar, M.A.M., Souleman, A.M., Buddrus, J., Bauer, H., Linscheid, M., 1984. Polyphenolic constituents of the flowers of *Tamarix nilotica*: the structure of Nilocitin, a new digalloylglucose. Tetrahedron Letters 25, 49.

Nogueira, E., Vassilieff, V.S., 2000. Hypnotic, anticonvulsant and muscle relaxant effects of *Rubus brasiliensis*. Involvement of $GABA_A$- system. Journal of Ethno-Pharmacology 70, 275.

Rauha, J., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., Vuorela, P., 2000. International Journal of Food Microbiology 56, 3.

Richard, R.M.E., Durham, D.C., Liu, X., 1994. Antibacterial activity of compounds from *Rubus pinfaensis*. Planta Medica 60, 471.

Shimomura, H., Sashida, Y., Adachi, T., 1988. Phenylpropanoid glucose esters from *Prunus buergeriana*. Phytochemistry 27, 641.

Tanaka, T., Tachibana, H., Nonaka, G., Nishioka, I., Hsu, F., Kohda, H., Tanaka, O., 1993. Tannins and related compounds. CXXII. New dimeric, trimeric and tetrameric ellagitannins, Lambertianins A-D, from *Rubus lambertianus* Sering. Chem. Pharm. Bull. 41, 1214.

Tanaka, T., Nonaka, G., Nishioka, I., 1991. Tannins and related compounds CIII. Isolation and characterization of new monomeric, dimeric and trimeric ellagitannins, Calamansanin and Calamanins A, B and C, from *Terminalia calamansanai*. Chem. Pharm. Bull. 39, 60.