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TRITERPENOID SAPONINS AND FLAVONOL GLYCOSIDES FROM *PHYTOLACCA THYRSIFLORA**

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Key Word Index—*Phytolacca thyrsiflora*; Phytolaccaceae; triterpenoid saponins; glycosylphytolaccagenins; glycosylserjanoates; glycosylspergulagenates; glycosylkaempferols; lignan; americanin A.

Abstract—The organs of *Phytolacca thyrsiflora* contain heterosides of different aglycones. In the roots only saponins based on phytolaccagenin and very probably on its de-*O*-methylderivative jaligonic acid have been located. The berries contain four new saponins based on serjanic acid. The de-*O*-methylanalogues, i.e. the spergulagenic acid derivatives, have so far been obtained only by saponification of the serjanoates. Finally, the leaves contain, besides glycosides of 7-*O*-methylkaempferol and of kaempferol, two of the new saponins based on serjanic acid. Only the known lignan, americanin A, was isolated from the seeds.

INTRODUCTION

Species of the genus *Phytolacca*, family Phytolaccaceae, superorder Caryophylliflorae *sensu* Dahlgren [1], are noted for their use in popular medicine against ailments of the joints (*P. acinosa*) [2], edema and rheumatism (*P. americana*, *P. insularis*)[3, 4] and dermatitis (*P. octandra*) [5]. Jointly with *P. bogotensis* [6], *P. dodecandra* [3, 7] and *P. rivinoides* [8] all contain triterpenoid saponins as the probable active principles. *P. dodecandra* has been found to be useful in the control of *Biomphalaria glabrata*, the snail-vector of bilharziasis [9].

Phytolacca thyrsiflora Fenzl ex Schmidt, popularly known as 'caruru bravo' or 'caruru selvagem', is used in Brazil for a series of alleged therapeutic properties [10]. It may also contain potential moluscicides and is known to intoxicate and sometimes even to kill cattle [11].

RESULTS AND DISCUSSION

All compounds isolated from the roots of *Phytolacca thyrsiflora* (Table 1) have been encountered previously in other species of the genus: phytolaccagenin-3-*O*-β-D-xylopyranoside (phytolaccoside-B, **1c**) and phytolaccagenin-3-*O*-β-D-glucopyranosyl (1→4)-β-D-xylopyranoside (phytolaccoside-E, **1e**) [12] in *P. americana* [13], as well as phytolaccagenin-3-*O*-β-D-glucopyranoside (**1d**) in *P. esculenta* [14]. All three saponins are based on phytolaccagenin (**1a**). This aglycone, jointly with jaligonic acid (**1b**), was also described in connection with *P. esculenta*. Both, **1a** and **1b**, were now obtained again by acid hydrolysis of a crude extract of the roots of *P. thyrsiflora*.

In contrast to the saponins of the roots, the saponins of the berries (**2d**, **4a**, **4b**, **4c**) and some of the saponins of the leaves (**2d**, **4c**) are all based on the well known serjanic acid (**2a**) as the aglycone. The evidence for this assertion is documented in Table 1. In serjanic acid position C-20 is occupied by a carbomethoxyl, while C-17 sustains a free carboxyl. These features continue in the heteroside **2d**, while the C-17 carboxyls of **4a**, **4b**, and **4c** are freed only by saponification and must originally have been part of an ester, predictably an *O*-glucosyl one. Saponification of the carbomethoxyls of all four compounds (**4a**, **4b**, **2d** and **4c**), leads respectively to **3b**, **3c**, **3e**, and **3f**. The sole concomitant change in the structure of the sugar units was observed for **2d**. Indeed **3e** contains one sugar unit less than its precursor (**2d**).

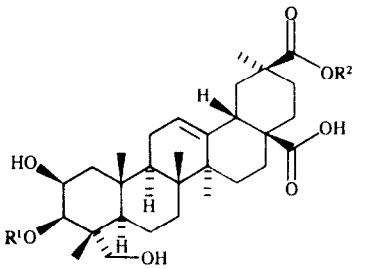
The number of sugar units per molecule was established via NMR evidence concerning the number of anomeric carbons (δ 102–107) and protons (δ 4.8–6.3) (Table 2), as well as through mass spectral data obtained by the FD and FAB techniques (Experimental). The nature of these units was determined via partial hydrolysis with dilute HCl (Table 1). For the triglycoside **2d** this led to the diglycoside **2c** and the monoglycoside **2b**; for **3f**, the saponification product of the triglycoside **4c**, this led to the diglycoside **3d** and the monoglycoside **3b**. The nature of the heterosidic sugar moieties was determined by ^{13}C NMR comparison with model compounds (Tables 3 and 4). This refers inclusively to the interosidic 1→3 bond between galactose and glucose of **2c** and **2d**, methyl-*O*-β-D-laminoribioside (**7**) [15] serving as model; and to D-allose, the terminal sugar unit of **4c** and **3f**, momordicoside F_2 (**12**) [16] serving as model. This was the only analytical procedure adopted in the characterization of allose. In all other cases sugars were freed and identified by chromatographic comparison with authentic samples. All saponins are glycosylated at C-3, as revealed by an 11 ppm downfield shift of the pertinent signals of the saponin with respect to the sapogenin.

Hydrolysis of the glycosylflavonoids **5c** and **5d** gave kaempferol (**5a**), while **5e** and **5f** gave 7-*O*-methylkaempferol (**5b**). Both flavonols are well known and, jointly

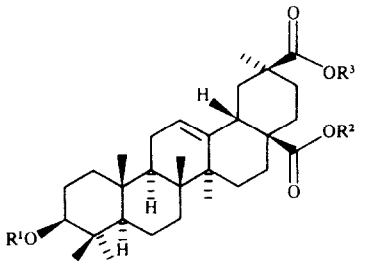
* Taken in part from the Doctorate thesis presented by M. H. to Universidade de São Paulo (1986).

Table 1. Constituents of *Phytolacca thyrsiflora* and of their reaction products

		Reagents		
		20% KOH	0.5 N HCl	1 N HCl
Root	BuOH extract			**1a**, **1b**
	1c			**1a**, xylose
	1d			**1a**, glucose
	1e			**1a**, xylose, glucose
Berry	BuOH extract			**2a**
	4a	**3b**		**2a**, glucose
	4b	**3c**		**2a**, glucose
	2d	**3e**	**2b**,**2c**	**2a**, glucose, galactose
	4c	**3f**	**3a**, **3b**, **3d**	**2a**, glucose
Leaf	**2d**			
	4c			
	5c			**5a**, galactose, xylose
	5e			**5b**, galactose, xylose
	5d			**5a**, galactose, glucose
	5f			**5b**, galactose, glucose
Seed	**6**			



1a R^1 = H, R^2 = Me
1b R^1 = R^2 = H
1c R^1 = Xyl(1–, R^2 = Me
1d R^1 = Glc(1–, R^2 = Me
1e R^1 = Glc(1→4)Xyl(1–, R^2 = Me



2a R^1 = R^2 = H, R^3 = Me
2b R^1 = Glc(1–, R^2 = H, R^3 = Me
2c R^1 = Gal(1→3)Glc(1–, R^2 = H, R^3 = Me
2d R^1 = Gal(1→2)Gal(1→3)Glc(1–, R^2 = H, R^3 = Me
3a R^1 = R^2 = R^3 = H
3b R^1 = Glc(1–, R^2 = R^3 = H
3c R^1 = Glc(1→2)Glc(1–, R^2 = R^3 = H
3d R^1 = Glc(1→4)Glc(1–, R^2 = R^3 = H
3e R^1 = Gal(1→3)Glc(1–, R^2 = R^3 = H
3f R^1 = All(1→4)Glc(1→4)Glc(1–, R^2 = R^3 = H
4a R^1 = R^2 = Glc(1–, R^3 = Me
4b R^1 = Glc(1→2)Glc(1–, R^2 = Glc, R^3 = Me
4c R^1 = All(1→4)Glc(1→4)Glc(1–, R^2 = Glc, R^3 = Me

with the carbohydrates indicated in Table 1, were identified with ease. Compound **5c** was identified by ^{13}C NMR (Table 5) with kaempferol-3-*O*[*β*-D-xylopyranosyl (1→2)-*β*-D-galactopyranoside] isolated previously from *Armoracia rusticana* [17]. The compound served as a model in the identification of the remaining flavonoids, including the disaccharide portion of **5c**. The identities of the analogous portions of compounds **5d** and **5f** were established using quercetin 3-*O*-[*β*-D-glycopyranosyl (1→2)-*β*-D-galactopyranoside] (**5g**) [18] as model.

The spectral data obtained in the present study for **5c** were previously recorded for an uncompletely characterized constituent of *Lysichiton camtschatcence* [19]. We wish to suggest that this constituent also shows structure **5c**.

EXPERIMENTAL

Mps: uncorr. DCCC was performed using the DCC-A apparatus of Tokyo Rikakikai, Tokyo (Japan); 300 tubes were used, upper layer (water layer) as stationary phase, in descending mode. FABMS and FDMS: JEOL JMS DX- 300/JMA-3500 system. In FABMS, the samples were dissolved in a glycerol matrix and the target was bombarded with Xe atoms. ^{1}H NMR spectra were measured at 400 MHz (JEOL GX-400) and 60 MHz (Varian EM-360) and ^{13}C NMR spectra at 20 MHz (Varian FT-80A).

Isolation of the constituents. Material of *Phytolacca thyrsiflora* was collected in the Municipality of Cotia, São Paulo, and identified by Professor Sylvio Panizza, Universidade de São Paulo. Roots, berries, leaves and seeds were treated separately by the following general method. Plant material, freed of fat by maceration in petrol, was extracted exhaustively with EtOH. The filtered soln was evapd and the residue, in H_2O, was extracted exhaustively with *n*-BuOH saturated with H_2O. The BuOH soln was evapd. Only the residue originating from seeds was at this stage extracted previously with EtOAc. All other residues were fractionated by successive column (50 × 4.5 cm) chromatography (silica gel H, eluant $CHCl_3$–MeOH–H_2O, 16:9:2) under pressure (N_2, 0.5 kgf/cm^2); and droplet countercurrent chromatography, descending mode, $CHCl_3$–

Table 2. NMR signals (δ, C_5D_5N) assigned to anomeric ^{13}C (20 MHz) and ^1H (400 MHz), coupling constants (J Hz) in parentheses

	^{13}C	^1H
2d	102.4, 104.9, 105.7	
3b	105.0, 106.6	4.97 (7.8)
3f	102.5, 104.9, 105.8	4.81 (7.6), 5.41 (7.0), 5.55 (7.8)
4a	106.8	4.96 (7.6), 6.33 (8.0)
4b	105.0, 105.8	4.93 (7.6), 5.41 (7.5), 6.33 (8.1)
4c	102.6, 105.0, 105.9	

Table 3. ^{13}C NMR chemical shifts of saponins in C_5D_5N and of model compounds (**7**, **8**, **9**) [In the first four lines only diagnostically relevant values are given, for other values relevent to the aglycone moieties see **4a** (Experimental)]

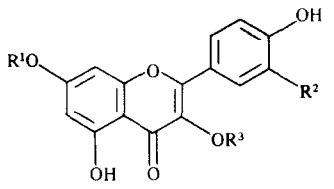
C	**2b**	**2c**	**2d**	**3b**	**3e**	**4a**	**7**	**8**	**9**
3	89.2	89.2‡	89.1	89.4	89.3	89.2			
28	180.2	180.0	179.8	180.7	180.4	176.3			
30	177.4	177.4	177.2	180.1	179.9	177.2			
OMe	51.9	51.7	51.7	—	—	51.9	56.5	56.7	56.6
C_3-*O*-Inner sugar									
1	106.9	106.3	105.7	106.8	106.3	106.8	105.5	105.4	106.1
2	75.8	74.6	75.0	75.3	74.7	75.8	73.8	74.8	72.5
3	78.7*	89.0‡	89.1	78.7*	88.7	78.5*	88.7	78.1	75.2
4	71.9	70.2§	70.9	72.0	70.0§	71.9	70.2	71.4	70.1
5	78.3*	77.8*	77.8*	78.2*	77.8*	78.2*	77.7	78.1	76.8
6	63.1	62.7†	61.9†	63.1	62.3	63.1	62.8	62.5	62.3
Intermediate sugar									
1			102.4						
2			82.8						
3			73.1						
4			69.9						
5			77.5*						
6			62.3†						
Terminal sugar									
1		106.3	104.9		106.3		105.0		
2		73.1	72.7		73.0		75.4		
3		75.1	74.5		75.2		78.3*		
4		70.0§	70.1		70.3§		71.9		
5		77.4*	77.4*		77.4*		78.2*		
6		62.3†	61.9†		62.3		62.8		
C_{28}-*O*-Inner sugar									
1						95.8			
2						75.8			
3						79.2			
4						71.1			
5						78.8*			
6						62.1			

7 Methyl-*O*-β-D-laminoribioside in C_5D_5N [15].
8 Methyl-*O*-β-D-glucopyranoside in C_5D_5N [15].
9 Methyl-*O*-β-D-galactopyranoside in C_5D_5N [15].
*,†,§,‡ Assignments may be interchanged between the carbons in the same column.

Table 4. ^{13}C NMR chemical shifts of saponins in C_5D_5N and of model compounds (**8**, **10**, **11**, **12**) [in the first four lines only diagnostically relevant values are given for other values respective to the aglycon moieties see **4a** (Experimental)]

C	3c	3d	3f	4b	4c	8	10	11	12
3	89.5	89.4	89.4	89.2	89.2				
28	180.5	180.6	179.6	176.2	176.5				
30	180.0	180.2	180.2	177.1	177.3				
OMe	—	—	—	51.8	52.1	56.7		58.9	
C_3-O-Inner sugar									
1	105.7	106.5	105.8	105.8	105.9	105.4	95.8	104.5	103.8
2	83.0	75.2§	75.2	83.1	75.1	74.8	82.8	74.2*	76.1
3	78.4*	76.9†	78.3	78.4*	78.2	78.1	77.2	76.4	72.4
4	71.6†	81.7	82.9	71.7	82.9	71.4	71.7	80.3	69.2
5	78.1*	76.4†	77.2	78.2*	77.2	78.1	77.2	75.9	73.0
6	62.9	62.4‡	62.1	62.9†	62.1	62.5	62.4	61.8	62.3
Intermediate sugar									
1			104.9		105.0				
2			74.7		74.5				
3			77.8*		78.2				
4			82.9		82.9				
5			77.7*		77.8				
6			62.1		62.1				
Terminal sugar									
1	105.1	105.0	102.5	105.0	102.6		103.9	103.9	
2	75.3	75.4§	75.8	74.3	75.9		74.9	74.6*	
3	78.4*	78.4*	72.9	78.0*	72.9		77.2	77.5	
4	71.9†	71.7	70.0	71.7	70.1		71.1	71.2	
5	77.0	78.3*	73.0	77.0	73.0		77.2	77.2	
6	62.9	62.5‡	62.4	62.6†	62.1		62.4	62.4	
C_{28}-O-Inner sugar									
1				95.8	95.9				
2				74.1	74.2				
3				79.2	79.3				
4				71.1	71.2				
5				78.8	78.9				
6				62.0	62.4				

10 β-Sophorose in D_2O [15].
11 Methyl-*O*-β-D-cellobioside in D_2O [15].
12 Momordicoside F_2 in C_5D_5N [16].
*,†,§,‡ Assignments may be interchanged between the carbons in the same column.



5a $R^1 = R^2 = R^3 = H$
5b R^1 = Me, $R^2 = R^3 = H$
5c $R^1 = R^2 = H$, R^3 = Xyl(1→2)Gal(1–
5d $R^1 = R^2 = H$, R^3 = Glc(1→2)Gal(1–
5e R^1 = Me, R^2 = H, R^3 = Xyl(1→2)Gal(1–
5f R^1 = Me, R^2 = H, R^3 = Glc(1→2)Gal(1–
5g R^1 = H, R^2 = OH, R^3 = Glc(1→2)Gal(1–

MeOH–H_2O, 7:13:8, rate 10–20 ml/hr. Yields: dry roots (450 g) gave BuOH extract (19.5 g): **1c** (190 mg), **1d** (110 mg) and **1e** (450 mg). Fresh roots (3.4 kg) gave BuOH extract (3.2 g): **1c** (30 mg), **1d** (10 mg) and **1e** (40 mg). Dry berries (without seeds) (138 g) gave BuOH extract (8.5 g): **2d** (1.50 g), **4a** (350 mg), **4b** (720 mg) and **4c** (2.3 g). Dry leaves (700 g) gave BuOH extract (29 g): **2d** (970 mg), **4c** (3.87 g), **5c** (390 mg), **5d** (510 mg), **5e** (250 mg) and **5f** (1.01 g). Seeds (134 g) gave EtOAc extract (7.2 g): **6** (840 mg).

Identifications. Phytolaccagenin (**1a**) [3,12], jaligonic acid (**1b**) [20], phytolaccoside B (**1c**), phytolaccoside E (**1e**) [12], serjanic acid (**2a**) [21], spergulagenic acid (**3a**) [3] and kaempferol-3-*O*-[β-D-xylopyranosyl (1→2)-β-D-galactopyranoside] (**5c**) [17], kaempferol-3-*O*-[β-D-glucopyranosyl (1→2)-β-D-galactopyranoside (**5d**) [22] and americanin A (**6**) [23] were identified by spectral comparisons with published data. Only com-

Table 5. ^{13}C NMR chemical shifts of glycosylflavonoids (**5d**, **5e**, **5f**) and of model compounds (**5c** and **5g**) in DMSO-d_6

Structural parts	Carbon	**5c** [17]	**5d**	**5e**	**5f**	**5g** [18]
Flavonol	2	156.9	155.8	155.8	156.2	156.2
	3	134.3	133.1	133.4	133.4	133.0
	4	178.8	177.7	177.7	177.8	177.3
	4a	105.2	104.4	105.0	105.1	104.1
	5	160.7	161.5	161.1	161.2	161.0
	6	99.5	99.0	98.5	98.4	98.6
	7	163.3	164.4	165.2	165.2	164.1
	8	95.1	93.9	92.4	92.4	93.4
	8a	157.9	156.5	156.4	156.4	155.4
	OMe	—	—	56.2	56.2	—
	1′	122.0	121.1	121.0	121.0	121.1
	2′	131.8	131.2	131.2	131.2	115.3
	3′	115.8	115.5	115.4	115.5	144.7
	4′	159.3	160.2	160.3	160.3	148.3
	5′	115.8	115.5	115.4	115.5	115.8
	6′	131.8	131.2	131.2	131.1	122.1
Inner Sugar	1″	100.8	98.7	98.0	98.6	98.4
	2″	78.9	80.6	79.9	80.6	80.7
	3″	74.0	73.5	73.8	73.6	73.2
	4″	69.4	67.9	68.0	67.8	67.4
	5″	74.4	76.0	76.1	75.9	75.8
	6″	61.1	61.1*	60.1	61.2*	59.8*
Terminal sugar	1‴	104.2	104.1	104.8	104.4	104.2
	2‴	75.8	74.6	74.1	74.6	74.3
	3‴	75.4	76.8	76.4	77.2	76.7
	4‴	70.1	70.0	69.6	70.0	69.5
	5‴	66.0	77.2	65.9	76.8	76.4
	6‴	—	60.2*	—	60.1*	60.6*

* Assignments may be interchanged between the carbons in the same column.

plementary, previously unpublished, data on these compounds are given below.

Phytolaccoside B (**1c**). FDMS *m/z* (rel. int.): 664 $[M]^+$, 618 $[M-H_2CO_2]^+$, 532 $[M-132]^+$, 133 $[Xyl+H-H_2O]^+$.

Phytolaccagenin-3-O-*β*-D-*glucopyranoside* (**1d**). Mp 215–217°. IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH), 1730 (ester CO), 1630, 1200–100 (C–O–C), 820. FDMS *m/z*: 733 $[M+K]^+$, 717 $[M+Na]^+$, 695 $[M+H]^+$, 694 $[M]^+$, 532 $[M-162]^+$, 163 $[Glc+H-H_2O]^+$. ^{13}C NMR: *δ* sugar moiety: 105.3, 75.2, 78.3, 71.2, 77.9, 62.3 (glucosyl C-1 to C-6).

Phytolaccoside E (**1e**). FDMS *m/z*: 873 $[M+2Na+H]^+$, 865 $[M+K]^+$, 849 $[M+Na]^+$, 826 $[M]^+$, 664 $[M-162]^+$, 532 $[M-162-132]^+$.

Serjanic acid-3-O-(*β*-D-*glucopyranoside*) (**2b**). Mp 195–198°. ^{13}C NMR: Table 3.

Serjanic acid-3-O-[*β*-D-*galactopyranosyl* (1→3)-*β*-D-*glucopyranoside*] (**2c**). Mp 217–219°. ^{13}C NMR: Table 3.

Serjanic acid-3-O-[*β*-D-*galactopyranosyl* (1→2)-*β*-D-*galactopyranosyl* (1→3)-*β*-D-glucopyranoside] (**2d**). Mp 203–206°. ^{13}C NMR: Table 3. FDMS *m/z*: 1064 $[M+2K]^+$, 1026 $[M+H+K]^+$, 1010 $[M+H+Na]^+$, 864 $[(M+H+K)-162]^+$, 848 $[(M+H+Na)-162]^+$, 525 $[(M+2H+Na)-3(162)]^+$.

Spergulagenic acid (**3a**). ^{13}C NMR: 39.1 (C-1), 28.0 (C-2), 78.3 (C-3), 39.8 (C-4), 56.0 (C-5), 18.8 (C-6), 33.4 (C-7), 39.4 (C-8), 48.2 (C-9), 37.5 (C-10), 23.9 and 24.0 (C-11, C-16), 123.5 (C-12), 144.9 (C-13), 42.2 (C-14), 28.6 (C-15), 46.5 (C-17), 43.5 (C-18), 43.2 (C-19), 44.2 (C-20), 31.2 (C-21), 34.8 (C-22), 28.9 (C-23), 16.6 (C-24), 15.6 (C-25), 16.6 (C-26), 26.3 (C-27), 180.0 and 180.5 (C-28, C-30), 29.2 (C-29).

Spergulagenic acid-3-O-(*β*-D-*glucopyranoside*) (**3b**). Mp 251–255° FABMS *m/z* : 647 $[M-H]^-$, 485 $[M-162]^-$. ^1H NMR (400 MHz): *δ* 4.97 (1H, *d*, *J* = 7.8 Hz, Glc H-1). ^{13}C NMR: Table 3.

Spergulagenic acid-3-O-[*β*-D-*glucopyranosyl* (1→2)-*β*-D-*glucopyranoside*] (**3c**). ^{13}C NMR. Table 4.

Spergulagenic acid-3-O-[*β*-D-*glucopyranosyl* (1→4)-*β*-D-*glucopyranoside*] (**3d**). Mp 224–227°. ^{13}C NMR: Table 4.

Spergulagenic acid-3-O-[*β*-D-*galactopyranosyl*(1→3)-*β*-D-*glucopyranoside*] (**3e**). Mp 228–231°. ^{13}C NMR: Table 3.

Spergulagenic acid-3-O-[*β*-D-*allopyranosyl* (1→4)-*β*-D-*glucopyranosyl* (1→4)-*β*-D-*glucopyranoside*] (**3f**). Mp 228–240°. FABMS *m/z* 972 $[M]^-$, 809 $[M-H-162]^-$, 647 $[M-H-2\ (162)]^-$, 458 $[M-H-3\ (162)]$. ^1H NMR (400 MHz): *δ* 5.55 (1H, *d*, *J* = 7.9 Hz, H-1 of sugar moiety), 5.41 (1H, *d*, *J* = 7.0 Hz, H-1 of sugar moiety), 4.81 (1H, *d*, *J* = 7.8 Hz, H-1 of sugar moiety). ^{13}C NMR: Table 4.

Serjanic acid-3-O-(*β*-D-*glucopyranoside*)-28-O-*β*-D-*glucopyranoside* (**4a**). Mp 194–198°. FABMS *m/z*: 823 $[M-H]^-$, 661 $[M-H-162]^-$, 499 $[M-H-2(162)]^-$. ^1H NMR (400 MHz): *δ* 6.33 (1H, *d*, *J* = 8.1 Hz, Glc H-1), 4.96 (1H, *d*, *J* = 7.6, Glc H-1). ^{13}C NMR: Table 3 and 38.8 (C-1), 26.8 (C-2), 39.6 (C-3), 56.0 (C-5), 18.6 (C-6), 33.3 (C-7), 40.0 (C-8), 48.1 (C-9), 37.1 (C-10), 23.7 and 23.9 (C-11, C-16), 124.0 (C-12), 144.0 (C-13), 42.2 (C-14), 28.5 (C-15), 46.7 (C-17), 42.6 (C-18), 43.3 (C-19), 44.1 (C-20), 30.0 (C-

21), 34.0 (C-22), 28.5 (C-23), 17.1 and 17.6 (C-24, C-26), 15.7 (C-25), 26.2 (C-27), 28.5 (C-29). The mean maximal variation of these values for the aglycon (serjanic acid) parts of **2b–2d**, **3b–3f**, **4b**, **4c** is + 0.3 ppm.

Serjanic acid-3-O-[*β*-D-glucopyranosyl (1→2)-*β*-*glucopyranoside*] 28-O-*β*-*glucopyranoside* (**4b**). Mp 209–212°. ^{13}C NMR: Table 4. FABMS *m/z*: 985 $[M-H]^-$, 823 $[M-H-162]^-$, 661 $[M-H-2](162)]^-$, 499 $[M-H-3(162)]^-$. ^{1}H NMR (400 MHz): δ 6.33 (1H, *d*, *J* = 8.0 Hz, Glc H-1), 5.40 (1H, *d*, *J* = 7.5 Hz, glc H-1), 493 (1H, *d*, *J* = 7.6 Hz, Glc H-1).

Serjanic acid-3-O-[*β*-D-*allopyranosyl*(1→4)-*β*-D-*glucopyranosyl* (1→4)-*β*-D-*glucopyranoside*] 28-O-*β*-D-*glucopyranoside* (**4c**). Mp 225–228°. FDMS *m/z*: 1188 $[M+H+K]^+$, 1172 $[M+H+Na]^+$, 1148 $[M]^+$, 1026 $[(M+H+K)-162]^+$, 1010 $[(M+H+Na)^-162]^+$, 864 $[(M+K)-324]^+$, 846 $[(M+Na)-324]^+$, 700 $[(M+K)-486]^+$, 684 $[(M+Na)-486]^+$, 540 $[(M+H+K)-648]^+$. ^{13}C NMR: Table 4.

Kaempferol-3-O-*β*-D-*glucopyranosyl* (1→2)-*β*-D-*galactopyranoside* (**5d**). FDMS *m/z*: 610 $[M]^+$, 448 $[M-162]^+$, 286 [genin]$^+$. ^{13}C NMR: Table 5.

Kaempferol-7-O-*methyl*-3-O-[*β*-D-*xylopyranosyl* (1→2)-*β*-D-*galactopyranoside*] (**5e**). Mp 168–171°. UV λ_{max}^{EtOH} nm: 268, 351; + NaOAc: 277, 400; + $AlCl_3$: 276, 303, 351, 396. FDMS *m/z*: 594 $[M]^+$, 462 $[M-132]^+$, 300 [genin]$^+$, 162 $[Gal+H-H_2O]^+$, 133 $[Xyl+H-H_2O]^+$. ^{1}H NMR (60 MHz, DMSO-d_6): δ 8.27 (2H, *d*, *J* = 8 Hz, H-2′ and H-6′), 6.95 (2H, *d*, *J* = 8 Hz, H-3′ and H-5′), 6.78 (1H, *d*, *J* = 2 Hz, H-8), 6.42 (1H, *d*, *J* = 2 Hz, H-6), 3.87 (3H, *s*, Me). ^{13}C NMR: Table 5.

Kaempferol-3-O-[*β*-D-*glucopyranosyl*(1→2)-*β*-D-*galactopyranoside*]-7-O-*methyl* (**5f**). Mp 175–178°. UV λ_{max}^{EtOH} nm: 267, 351; + NaOAc: 269, 399; + $AlCl_3$: 278, 306, 354, 404. FDMS *m/z*: 647 $[M+Na]^+$, 625 $[M+H]^+$, 462 $[M-162]^+$, 300 [genin]$^+$. ^{1}H NMR (60 MHz, DMSO-d_6): δ 8.15 (2H, *d*, *J* = 8 Hz, H-2′ and H-6′), 6.80 (2H, *d*, *J* = 8 Hz, H-3′ and H-5′), 6.73 (1H, *d*, *J* = 2 Hz, H-8), 6.40 (1H, *d*, *J* = 2Hz, H-6), 3.88 (3H, *s*, Me). ^{13}C NMR: Table 5.

Hydrolysis with 1 *N* HCl. The saponins (50 mg) in EtOH (2 ml) and 1 N HCl (2 ml) were heated under reflux (4 hr). The EtOH was evapd. The residual aq. solns were poured on ice-H_2O. The ppt. was collected and recrystallized from EtOH. The mother liquors were passed through an ion exchange column (Amberlite IRA-45) to neutralise and then evapd. The residues were submitted to standard paper (Whatman no. 1) chromatography (BAW 4:1:5). Detection of spots relied on the application of aniline hydrogen phthalate and heating (110°, 10 min). The identification of sugars involved cochromatography with authentic samples.

Hydrolysis with alkali. The saponins (100 mg each) in MeOH containing 20% KOH (60 ml) were heated under reflux (10 hr). The cooled mixtures were poured on ice-H_2O, acidified with 4 N HCl until pH 5–6 and extracted with *n*-BuOH. The BuOH layer was separated and evapd. The residues were fractionated by CC (silica gel H, $CHCl_3$–MeOH–H_2O, 16:9:2) under pressure. Yields: **2d** → **3e** (21 mg), **4a** → **3b** (15 mg), **4b** → **3c** (13 mg), **4c** → **3f** (10 mg).

Hydrolysis with 0.5 *N HCl*. The saponins (70 mg each) in EtOH (20 ml) and 0.5 N HCl (20 ml) were heated under reflux (2 hr). The cooled mixtures were neutralized with aq. 1 N NaOH. The EtOH was evapd and the residue dissolved in H_2O. The soln was extracted with *n*-BuOH saturated with H_2O. The BuOH layer was evapd. The residue was submitted to a sequence of chromatographic procedures up to the isolation of pure products of partial hydrolysis. The aq. layer was examined for sugars by the traditional paper chromatographic method (BAW, 4:1:5). Yields: **2d** → **2b** (21 mg) and **2c** (11 mg), **3f** → **3a** (10 mg), **3b** (15 mg) and **3d** (7 mg).

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