

FOUR ACYLATED FLAVONOL GLYCOSIDES FROM LEAVES OF *STRYCHNOS VARIABILIS**[†]

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Abstract—Four new acylated flavonol glycosides have been isolated and identified from the leaves of *Strychnos variabilis*: quercetin 3-(4'-*trans*-*p*-coumaroyl)robinobioside-7-glucoside (variabiloside A) and its *cis* derivative (variabiloside B), kaempferol 3-(4'-*trans*-*p*-coumaroyl)robinobioside-7-glucoside (variabiloside C) and its *cis* derivative (variabiloside D).

INTRODUCTION

In an earlier paper [1] we reported a mixture of flavonol glycosides from the leaves of *Strychnos variabilis* Wildem. from which we later isolated two unusual compounds: quercetin and kaempferol 3-robinobiosides [2]. The remaining two isolates were, mixtures of quercetin and kaempferol glycosides, each containing two acylated compounds. In the present study the glycosides of the first mixture were separated and their structures fully elucidated: the major component as variabiloside A (1) and the minor one as variabiloside B (2). It was not possible to separate the glycosides of the other mixture. Nevertheless the major component, variabiloside C (3) was clearly identified. The minor component, variabiloside D (4), which was not detected in the spectra of the mixture, was identified from a study of the hydrolysis product after treatment with β -glucosidase.

As far as we know, such complex *p*-coumaroyl triglycosides have not previously been described. A few other complex *p*-coumaroyl triglycosides have already been isolated [3-5] but their structures have not been fully elucidated. The variabilosides of *Strychnos variabilis* may be of pharmaceutical interest because *p*-coumaroyl derivatives of quercetin and kaempferol diglycosides are the major components of the pharmaceutical extract of *Ginkgo biloba* [6, 7] which has a significant effect on the symptoms of cerebrovascular insufficiency and poor arterial circulation [8].

RESULTS AND DISCUSSION

Characterisation of variabilosides A and C

Acidic hydrolysis afforded galactose, glucose, rhamnose and the aglycones: quercetin from 1 and kaempferol from 3. The UV spectra showed an unusual band at 316 nm due to a *p*-coumaroyl acid chromophore (see below).

Compounds 1 and 3 on hydrolysis with β -glucosidase gave the new products 5 and 6, respectively, which both only yielded galactose and rhamnose upon acidic hydrolysis. Compounds 5, 6, 1 and 3 were submitted to alkaline hydrolysis [9] and gave respectively quercetin 3-rhamnosylgalactoside 7, kaempferol 3-rhamnosylgalactoside 8, quercetin 3-rhamnosylgalactoside-7-glucoside 9 and kaempferol 3-rhamnosylgalactoside-7-glucoside 10. These structures were identified from UV spectral data. Mild acidic hydrolysis [10] of 7 and 8 indicated that the galactose was attached to the aglycone.

Alkaline hydrolysis also afforded *p*-coumaric acid which must be attached to the rhamnosylgalactoside unit because the UV spectra of 5 and 6 like those of 1 and 3 exhibited a band at 316 nm. When we compared the ^1H NMR spectra of 1 and 5, we observed that 1 had an additional doublet at 5.08 ppm with a large coupling constant (7 Hz): this was the anomeric proton ($\text{H}-1''$) of β -glucose; the chemical shift confirmed the position of linkage between sugar and aglycone at C-7 [11]. The doublet at 5.49 ppm with large coupling constant (7 Hz) was assigned to the anomeric proton ($\text{H}-1''$) of β -galactose and confirmed linkage at C-3. The chemical shift of the anomeric proton ($\text{H}-1''$) of α -rhamnose was identical with that of the rhamnose from quercetin 3-robinobioside [2] and indicated a (1 \rightarrow 6) linkage between rhamnose and galactose. The anomeric proton ($\text{H}-1''$) of galactose exhibited a small downfield shift when compared with quercetin 3-robinobioside [2], indicating that the acyl group was linked to the galactose moiety. The linkage was confirmed by ^{13}C NMR spectra. The spectra also exhibited two doublets with large coupling constant (17 Hz): these showed the *trans* configuration of *p*-coumaric acid. Compound 3 has the same sugar substitution and a *trans*-*p*-coumaroyl unit.

Comparison of the ^{13}C NMR spectra of 1 and 5 allowed easy recognition of the 7-*O*- β -D-glucopyranosyl unit (Table 1) [12]. The shifts of the rhamnose signals indicated an α -L-rhamnopyranosyl unit and the C-1'' signal (100.4 ppm), a rhamnose not directly attached to the aglycone. The shift of the C-1'' signal for galactose was

* Part 2 in the series 'Flavonol glycosides from leaves of *Strychnos variabilis*'. For Part 1, see ref. [2].

Table I. ^{13}C NMR data*.

C	Compound					
	1	2	5	7†	3	8†
2	156.1	156.1	156.4	156.4	157.2	156.5
3	133.5	133.4	133.3	133.6	133.5	133.3
4	177.6	177.7	177.5	177.4	177.6	177.5
5	161.0	161.0	161.3	161.2	160.9	161.2
6	99.5	99.5	98.8	98.8	99.5	98.8
7	163.0	163.0	164.3	164.4	163.0	164.0
8	94.6	94.6	93.7	93.6	94.7	93.8
9	157.2	157.6	156.6	156.4	156.1	156.5
10	105.7	105.7	104.0	103.8	105.7	103.8
1'	121.8	121.9	121.7	121.9	120.8	120.9
2'	115.3	115.4	115.2	115.2	131.1	131.0
3'	144.9‡	145.0	144.9‡	144.9	115.2	115.1
4'	149.1	148.9	148.7	148.6	160.3	160.0
5'	116.7	116.5	116.5	116.0	115.2	115.1
6'	121.0	121.0	121.2	121.1	131.1	131.0
1"	101.4	101.3	101.4	102.2	101.5	102.1
2"	71.8	71.8	71.8	71.1	71.7	71.1
3"	71.2	71.2	71.1	73.1	71.1	73.0
4"	70.0	69.9	69.9	68.1	70.0	68.1
5"	71.5	71.5	71.5	73.6	71.6	73.6
6"	64.9	65.0	64.8	65.2	64.9	65.4
1'''	100.4	100.4	100.4	100.1	100.4	100.1
2'''	70.4‡	70.4‡	70.4‡	70.5‡	70.4‡	70.4‡
3'''	70.4‡	70.6‡	70.5‡	70.7‡	70.5‡	70.7‡
4'''	72.0	71.9	71.9	72.0	72.0	72.0
5'''	68.4	68.6	68.5	68.3	68.4	68.3
6'''	17.9	17.8	17.8	17.9	17.8	17.9
1''''	99.9	100.0			100.0	
2''''	73.2	73.2			73.2	
3''''	77.3	77.3			77.2	
4''''	69.7	69.7			69.7	
5''''	76.4	76.5			76.5	
6''''	60.7	60.7			60.7	
1'''''	125.3	125.6	125.2		125.1	
2'''''	130.4	133.1	130.4		130.2	
3'''''	116.0	115.0	116.0		115.9	
4'''''	159.9	158.9	159.8		159.9	
5'''''	116.0	115.0	116.0		115.9	
6'''''	130.4	133.1	130.4		130.2	
7'''''	145.1‡	143.9	145.0‡		144.8	
8'''''	114.3	115.0	114.3		114.3	
9'''''	166.1	165.2	166.1		165.9	

* ^{13}C NMR (75.5 MHz, DMSO- d_6).

† Data from [2].

‡ Assignments may be reversed.

that of a 3-*O*- β -D-galactopyranosyl unit. The downfield shift of the C-6" (4.6 ppm) was due to the rhamnosylation and confirmed a (1 → 6) linkage between the sugars. The C-5" and C-3" signals were shifted upfield (2 ppm) while the C-4" was shifted downfield (1.8 ppm) indicating that a trans-*p*-coumaric acid moiety was linked to C-4". The shifts of trans-*p*-coumaric acid were in agreement with published data [6, 12]. Thus **1** is quercetin 3-rhamnosyl (1 → 6)(4"-trans-*p*-coumaroyl)galactoside-7-glucoside. The sugar and *p*-coumaroyl moieties of **3** were identical with those of **1**. Thus, **3** is kaempferol 3-rhamnosyl(1 → 6)(4"-trans-*p*-coumaroyl)galactoside-7-glucoside.

Characterisation of variabilosides B and D

Compounds **2** and **4** when submitted to β -glucosidase hydrolysis gave the new compounds **11** and **12**; respectively, which have higher R_f values than **5** and **6** (system 1). The UV spectra of **11** and **12** were similar to those of **5** and **6** but the band at 316 nm was smaller. Compounds **11** and **12** only yielded galactose and rhamnose upon acidic hydrolysis. Compounds **2**, **4**, **11** and **12** were submitted to alkaline hydrolysis and gave the same compounds as those obtained from **1**, **3**, **5** and **6** respectively. These structures were confirmed by UV spectroscopy. Alkaline hydrolysis

also gave a compound having lower R_f than *trans-p*-coumaric acid.

The ^1H NMR spectra of the sugar moieties of 2 and 12 were similar to those of 1 and 5. The spectra exhibited two doublets at 5.8 and 6.92 ppm with large coupling constant (13 Hz) and two doublets at 6.75 and 7.72 ppm with 9 Hz coupling constant. These signals were assigned to a *cis-p*-coumaroyl unit in agreement with published data [13]. The ^{13}C NMR spectrum of 2 is identical with that of 1 except for the *cis-p*-coumaroyl signals (Table 1). Compound 2 is thus quercetin 3-rhamnosyl(1 \rightarrow 6)(4"-*cis-p*-coumaroyl)galactoside-7-glucoside (Fig. 1) and 4 is kaempferol 3-rhamnosyl(1 \rightarrow 6)(*cis-p*-coumaroyl)galactoside-7-glucoside.

EXPERIMENTAL

Plant material. Leaves of *Strychnos variabilis* Wildem. were collected in 1951 at the Botanical Garden of Kisantu (Zaire) and stored sheltered from light, in the laboratorium of Pharmacognosy (Liège, University). Herbarium specimens are kept in the Botanical Garden of Belgium at Meise and in the University of Liège (Duvigneaud 147 et 725).

General techniques. TLC of glycosides were carried out on silicagel 60 F254 precoated plastic sheets Merck^R with EtOAc-HCOOH-H₂O (6:1:1) (Syst. 1) and on cellulose precoated plastic sheets Merck^R with HOAc-H₂O (3:17) (syst. 2); TLC of aglycones on cellulose with HOAc-H₂O (3:2), CHCl₃-HOAc-H₂O (10:9:1) and *n*-BuOH-HOAc-H₂O

(4:1:1). Glycosides and aglycones were visualized with amonoethylidiphenylborinate-PEG 400 [14]; TLC of sugars on silicagel 60 F254 with *n*-BuOH-Me₂CO-NaH₂PO₄ 1.6% in water (4:5:1) and visualized with aniline phthalate reagent. Coumaric acids were chromatographed on silicagel 60 F254 precoated plates with CH₂Cl₂-MeOH (4:1) and visualized with Paskova and Munk's reagent [15] (Table 2).

Isolation of flavonol glycosides. Leaves (100 g) were extracted with EtOH, the concd extract taken up in H₂O and successively extracted with Et₂O, EtOAc and *n*-BuOH. The crude BuOH extract (4.6 g) purified by LC (Lobar^R LichroPrep^R RP-8; 20–30% aq. Me₂CO) was submitted to DCCC with CHCl₃–MeOH–PrOH–H₂O (10:12:3:8) in the descending mode (300 columns, 40 cm × 2 mm, instrument DCC-A, Tokyo Rikakikai, Japan). Variabilosides A and B were separated by LC (Lobar^R (LichroPrep^R RP-8; 10–20% aq. Me₂CO). After β -glucosidase treatment, the hydrolysed glycosides were separated by DCCC (150 columns, 40 × 2 mm) with CHCl₃–MeOH–H₂O (5:6:4) in the descending mode. Finally, all the products were purified on Sephadex LH 20 column eluted with MeOH.

Quercetin 3-rhamnosyl (1 → 6) (4^o-trans-p-coumaroyl)galactoside-7-glucoside (1). Variabilioside A. UV λ_{max} nm: 316, 268 sh, 258; (NaOMe) 367, 266; (AlCl₃) 437, 315, 300, 277; (AlCl₃ + HCl) 403, 315, 300, 276; (NaOAc) 372, 325, 265; (NaOAc + H₃BO₃) 383, 317, 264. ¹H NMR (300 MHz, DMSO-d₆): δ 7.66, 7.63 (2H, H-2', H-6'), 7.54 (2H, d, J = 9 Hz, H-2 coum, H-6 coum), 7.52 (1H, d, J = 17 Hz, H-7 coum), 6.89 (1H, d, J = 9 Hz, H-5'), 6.83 (2H, d, J = 9 Hz, H-3 coum H-5 coum), 6.75 (1H, d, J = 2 Hz, H-8), 6.45 (1H, d, J = 2 Hz, H-6), 6.39 (1H, d, J = 17 Hz,

Table 2. Chromatographic data (see Experimental)

Compounds	Rf values		Fluorescence after spraying
	Syst 1	Syst 2	
1. Quercetin 3-(4"- <i>trans</i> - <i>p</i> -coumaroyl)robinobioside-7-glucoside (Variabiloside A)	0.24	0.56	orange red
2. Quercetin 3-(4"- <i>cis</i> - <i>p</i> -coumaroyl)robinobioside-7-glucoside (Variabiloside B)	0.24	0.56	orange red
3. Kaempferol 3-(4"- <i>trans</i> - <i>p</i> -coumaroyl)robinobioside-7-glucoside (Variabiloside C)	0.29	0.62	green
4. Kaempferol 3-(<i>cis</i> - <i>p</i> -coumaroyl)robinobioside-7-glucoside (Variabiloside D)	0.29	0.62	green
5. Quercetin 3-(4"- <i>trans</i> - <i>p</i> -coumaroyl)robinobioside	0.67	0.37	orange
6. Kaempferol 3-(4"- <i>trans</i> - <i>p</i> -coumaroyl)robinobioside	0.73	0.42	green
7. Quercetin 3-robinobioside	0.36	0.51	orange
8. Kaempferol 3-robinobioside	0.43	0.55	green
9. Quercetin 3-robinobioside-7-glucoside	0.07	0.65	orange red
10. Kaempferol 3-robinobioside-7-glucoside	0.09	0.74	green
11. Quercetin 3-(4"- <i>cis</i> - <i>p</i> -coumaroyl)robinobioside	0.73	0.37	orange
12. Kaempferol 3-(<i>cis</i> - <i>p</i> -coumaroyl)robinobioside	0.79	0.42	green

*See Experimental.

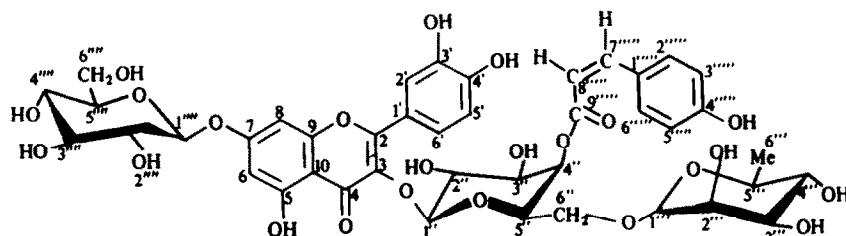


Fig. 1.

H-8 coum), 5.49 (1H, d, $J = 7$ Hz, H-1 gal), 5.08 (1H, d, $J = 7$ Hz, H-1 glc), 4.38 (1H, s, H-1 rha), 3.3 (m, sugar protons), 0.94 (3H, d, $J = 6$ Hz, Me rha).

Quercetin 3-rhamnosyl(1 → 6)(4"-trans-p-coumaroyl) galactoside (5). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 315, 266, 257; (NaOMe) 366, 271; (AlCl₃) 436, 309 300 sh, 277; (AlCl₃ + HCl) 402, 311, 300, 276; (NaOAc) 378, 320, 275; (NaOAc + H₃BO₃) 379, 315, 265. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.66, 7.63 (2H, H-2', H-6'), 7.54 (2H, d, $J = 9$ Hz, H-2 coum H-6 coum), 7.52 (1H, d, $J = 17$ Hz, H-7 coum), 6.88 (1H, d, $J = 9$ Hz, H-5'), 6.82 (2H, d, $J = 9$ Hz, H-3 coum H-5 coum), 6.4 (1H, d, $J = 2$ Hz, H-8), 6.38 (1H, d, $J = 17$ Hz, H-8 coum), 6.2 (1H, d, $J = 2$ Hz, H-6), 5.46 (1H, d, $J = 7$ Hz, H-1 gal), 4.38 (1H, s, H-1 rha), 3.3 (m, sugar protons), 0.93 (3H, d, $J = 6$ Hz, Me rha).

Quercetin 3-rhamnosyl(1 → 6)(4"-cis-p-coumaroyl) galactoside-7-glucoside (2). Variabiloside B. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 315, 267 sh, 257; (NaOMe) 368, 270; (AlCl₃) 440, 316 sh, 300, 276; (AlCl₃ + HCl) 402, 317 sh, 299, 275; (NaOAc) 380, 322, 264; (NaOAc + H₃BO₃) 383, 314, 263; ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.73 (2H, d, $J = 9$ Hz, H-2 coum H-6 coum), 7.63, 7.61 (2H, H-2', H-6'), 6.9 (1H, d, $J = 13$ Hz, H-7 coum), 6.83 (1H, d, $J = 9$ Hz, H-5'), 6.74 (2H, d, $J = 9$ Hz, H-3 coum H-5 coum), 6.73 (1H, H-8), 6.45 (1H, H-6), 5.9 (1H, d, $J = 13$ Hz, H-8 coum), 5.49 (1H, d, $J = 7$ Hz, H-1 gal), 5.06 (1H, d, $J = 7$ Hz, H-1 glc), 4.39 (1H, s, H-1 rha), 3.3 (m, sugar protons), 0.95 (3H, d, $J = 6$ Hz, Me rha).

Kaempferol 3-rhamnosyl(1 → 6)(4"-trans-p-coumaroyl) galactoside-7-glucoside (3). Variabiloside C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 316, 268; (MeONa) 368, 278 sh; (AlCl₃) 397, 320, 303, 277; (AlCl₃ + HCl) 396, 320, 302, 278; (NaOAc) 373, 324, 266; (NaOAc + H₃BO₃) 321, 267; ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.13 (2H, d, $J = 8$ Hz, H-2', H-6'), 7.54 (2H, d, $J = 9$ Hz, H-2 coum, H-6 coum), 7.52 (1H, d, $J = 17$ Hz, H-7 coum), 6.95 (2H, d, $J = 8$ Hz, H-3', H-5'), 6.82 (2H, d, $J = 9$ Hz, H-3 coum, H-5 coum), 6.8 (1H, s, H-8), 6.47 (1H, s, H-6), 6.35 (1H, d, $J = 17$ Hz, H-8 coum), 5.48 (1H, d, $J = 7$ Hz, H-1 gal), 5.08 (1H, d, $J = 7$ Hz, H-1 glc), 4.38 (1H, s, H-1 rha), 3.3 (m, sugar protons) 0.94 (3H, d, $J = 6$ Hz, Me rha).

Kaempferol 3-rhamnosyl(1 → 6)(cis-p-coumaroyl) galactoside (4). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 316, 267; (MeONa) 378, 275; (AlCl₃) 396, 305,

275; (AlCl₃ + HCl) 393, 306, 274; (NaOAc) 382, 315, 275; (NaOAc + H₃BO₃) 317, 267. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.04 (2H, d, $J = 8$ Hz, H-2', H-6'), 7.72 (2H, d, $J = 9$ Hz, H-2 coum, H-6 coum), 6.92 (1H, d, $J = 13$ Hz, H-7 coum), 6.88 (2H, d, $J = 8$ Hz, H-3', H-5'), 6.74 (2H, d, $J = 9$ Hz, H-3 coum, H-5 coum), 6.43 (1H, s, H-8), 6.21 (1H, s, H-6), 5.8 (1H, d, $J = 13$ Hz, H-8 coum), 5.44 (1H, d, $J = 7$ Hz, H-1 gal), 4.36 (1H, s, H-1 rha), 0.94 (3H, d, $J = 6$ Hz, Me rha).

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