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SIX FLAVONOL GLYCOSIDES FROM LEAVES OF *STRYCHNOS VARIABILIS**

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Key Word Index—*Strychnos variabilis*, Loganiaceae; acylated flavonol glycosides; ethyl acetate soluble flavonol glycosides: variabilosides E, F, G, and H; water soluble flavonol glycosides: quercetin and kaempferol 3-robinobioside-7-glucosides, DCCC.

Abstract—Four new acylated flavonol glycosides have been identified from leaves of *Strychnos variabilis*: quercetin 3-(4″-*trans*-*p*-coumaroyl) robinobioside and its *cis* derivative, kaempferol 3-(4″-*trans*-*p*-coumaroyl) robinobioside and its *cis* derivative. Quercetin and kaempferol 3-robinobioside-7-glucosides were also identified.

INTRODUCTION

We recently isolated four new acylated flavonol triglycosides from the butanol soluble fraction of the leaves of *S. variabilis* [1] (variabilosides A, B, C and D). From the ethyl acetate soluble fraction, we have now isolated four acylated diglycosides, variabilosides E (**1**), F (**2**), G (**3**) and H (**4**). As far as we know, such *p*-coumaroyl diglycosides have not previously been described. Furthermore, variabilosides B, D, F and H are the first *cis* derivatives of flavonols to be characterized. The first *p*-coumaroyl ester of a flavonol diglycoside was isolated from *Ginkgo biloba* [2]. A *cis* derivative was previously isolated from the petals of *Eustoma grandiflorum* [3] but its structure has only been partially elucidated. The variabilosides of *S. variabilis* might be of pharmaceutical interest because *p*-coumaroyl esters of quercetin and kaempferol diglycosides are the major components of the pharmaceutical extract of *Ginkgo biloba* [4], which has a significant effect on the symptoms of cerebrovascular insufficiency and poor arterial circulation [5]. Flavonoids are considered as a class of natural products of high pharmacological potency [6] but unfortunately, many of them have a low solubility in water. We have also isolated two flavonol glycosides very soluble in water: quercetin and kaempferol 3-robinobioside-7-glucoside (**5** and **6**). Similar compounds have been previously isolated from leaves of *Atropa belladonna* [7] but their structures have not been fully elucidated.

RESULTS AND DISCUSSION

Structure of variabilosides E and G

Acidic hydrolysis afforded galactose, rhamnose and aglycones: quercetin from **1** and kaempferol from **3**. The UV spectra showed an unusual band at *ca* 315 nm due to the *p*-coumaroyl unit (see below). Compounds **1** and **3** submitted to alkaline hydrolysis [8] gave *p*-coumaric acid and quercetin 3-rhamnogalactoside (**7**) and kaempferol 3-rhamnogalactoside (**8**) respectively (Table 1).

The ^{1}H NMR spectra of the sugar and *p*-coumaroyl moieties of **1** and **3** were similar. The spectra exhibited two doublets with large coupling constant (16 Hz) which showed the *trans* configuration of *p*-coumaric acid. The doublet at about 5.45 ppm with 7.5 Hz coupling constant was assigned to the anomeric proton (H-1″) of β-galactose and confirmed linkage at C-3 [9]. The chemical shift of the singlet for the anomeric proton of α-rhamnose (H-1‴) at about 4.38 ppm was identical with the one for the rhamnose of quercetin and kaempferol 3-robinobioside [10] and indicated a (1→6) linkage between rhamnose and galactose. The anomeric proton (H-1″) of galactose exhibited a small downfield shift (0.15 ppm) compared with quercetin and kaempferol 3-robinobioside [10] This indicated that the acyl group was linked to the galactose and was confirmed by ^{13}C NMR spectroscopy.

The ^{13}C NMR spectra of **1** and **3** (Table 2) showed the signals of a α-L-rhamnopyranosyl unit not directly attached to the aglycone. The shift of the C-1″ signal of galactose (101.5) was the one 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 (2.1 ppm) while the C-4″ signal was shifted downfield (1.7 ppm), indicating that *trans*-*p*-coumaroyl acid was linked to C-4″. The shifts for *trans*-*p*-coumaric acid were in agreement with published data [11]. Thus **1** is quercetin 3-rhamnosyl (1→6) (4″-*trans*-*p*-coumaroyl)galactoside and **3** is kaempferol 3-rhamnosyl (1→6) (4″-*trans*-*p*-coumaroyl)galactoside.

Structure of variabilosides F and H

Acidic hydrolysis afforded galactose, rhamnose and the aglycones: quercetin from **2** and kaempferol from **4**. The UV spectra of **2** and **4** were similar to those of **1** and **3** but the band at 315 nm was smaller. Compounds **2** and **4** submitted to alkaline hydrolysis [8] gave an acid with a

*Part 3 in the series 'Flavonol glycosides from leaves of *Strychnos variabilis*' For Part 1, see ref. [10] and for Part 2, ref [1]

Table 1 Chromatographic data for flavonol glycosides

Flavonol glycoside	R_f values* Syst 1	Syst 2	Fluorescence
1 Quercetin 3-(4″-*trans*-*p*-coumaroyl) robinobioside (variabiloside E)	0 67	0 37	orange
2 Quercetin 3-(4″-*cis*-*p*-coumaroyl) robinobioside (variabiloside F)	0 73	0 37	orange
3 Kaempferol 3-(4″-*trans*-*p*-coumaroyl) robinobioside (variabiloside G)	0 73	0 42	green
4 Kaempferol 3-(4″-*cis*-*p*-coumaroyl) robinobioside (variabiloside H)	0 79	0 42	green
5 Quercetin 3-robinobioside-7-glucoside	0 07	0 65	orange-red
6 Kaempferol 3-robinobioside-7-glucoside	0 09	0 74	green
7 Quercetin 3-robinobioside	0 36	0 51	orange
8 Kaempferol 3-robinobioside	0 43	0 55	green

*For system details see Experimental

lower R_f than *trans*-*p*-coumaric acid and quercetin-3-rhamnosylgalactoside (**7**) and kaempferol 3-rhamnosylgalactoside (**8**) respectively (Table 1)

The ^{1}H NMR spectra of the sugar moiety of **2** and **4** were similar to those of **1** and **3** The spectra exhibited two doublets at 5 9 and 6 91 ppm with a large coupling constant (13 Hz) and two doublets at 6 75 and 7 75 with an 8.5 Hz coupling constant The signals were assigned to a *cis*-*p*-coumaroyl unit in agreement with published data [12]

The ^{13}C NMR spectra of **2** and **4** were similar to those of **1** and **3** except for the *cis*-*p*-coumaroyl signals (Table 2). Compound **2** is thus quercetin 3-rhamnosyl (1→6) (4″-*cis*-*p*-coumaroyl)galactoside (Fig 1) and **4** is kaempferol 3-rhamnosyl (1→6) (4″-*cis*-*p*-coumaroyl)galactoside

Structure of water soluble flavonol glycosides

Acidic hydrolysis afforded galactose, glucose, rhamnose and the aglycones quercetin from **5** and kaempferol from **6** The UV spectra showed no unusual bands and that the 3-and 7-hydroxyl groups were substituted Compounds **5** and **6** submitted to β-glucosidase hydrolysis gave quercetin 3-robinobioside (**7**) and kaempferol 3-robinobioside (**8**) respectively (Table 1). The ^{1}H NMR spectra showed signals for the anomeric protons of β-galactose (H-1″) and β-glucose (H-1‴) linked at C-3 and C-7, respectively The chemical shift of the anomeric proton of α-rhamnose (H-1‴) was similar to that of quercetin and kaempferol 3-robinobioside [10] and indicated a (1→6) linkage between the sugars. The ^{13}C NMR spectra showed signals for 3-*O*-β-D-galactopyranose, 7-*O*-β-D-glucopyranose and α-L-rhamnopyranose not directly attached to the aglycone The downfield shift (5 2 ppm) of the C-6″ signal of galactose was due to rhamnosylation and confirmed a (1→6) linkage between the sugars

EXPERIMENTAL

Plant material Leaves of *Strychnos variabilis* were collected in 1951 at the Botanical Garden of Kisantu (Zaire) and dark-stored 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 silica gel 60 F 254 precoated plastic sheets Merck® with EtOAc–HCO_2H–H_2O (6 1 1) (syst 1) and on cellulose precoated plastic sheets Merck® with HOAc–H_2O (3 17) (syst 2), TLC of aglycones on cellulose with HOAC–H_2O (3 2), $CHCl_3$–HOAc–H_2O (10 9 1) and *n*-BuOH–HOAc H_2O (4 1 1) Glycosides and aglycones were visualized with aminoethyldiphenylborinate-PEG 400 [13], TLC of sugars on silica gel 60 F 254 with *n*-BuOH–Me_2CO–NaH_2PO_4 1 6% in H_2O (4 5 1) and visualized with aniline phthalate reagent, TLC of coumaric acids on silica gel 60 F 254 precoated plates with CH_2Cl_2–MeOH (4 1) and visualized with Paskova and Munk's reagent [14]

Isolation Leaves (100 g) were extracted with EtOH and the concd extract taken up in hot water The filtrate was successively extracted by Et_2O, EtOAc and *n*-BuOH The residual aq extract (4 7 g) was purified by LC (Lobar® LichroPrep® RP-8, 10% aq Me_2CO and then Sephadex® LH 20 column, H_2O), and finally freeze-dried The crude EtOAc extract purified by LC (Lobar® LichroPrep® RP-8, 30–50% aq Me_2CO) was submitted to DCCC with ($CHCl_3$–MeOH–H_2O (5 6 4) in the descending mode (150 columns, 40 × 2 mm, instrument DCC-A, Tokyo Rikakikai, Japan) Finally, the variabilosides were purified on a Sephadex® LH 20 column eluted with MeOH

Quercetin 3-rhamnosyl (1→6)(4″-trans-p-coumaroyl)-galactoside (variabiloside E) UV λ_{max}^{MeOH} nm 314, 266 257, (NaOMe) 368, 271, ($AlCl_3$) 437, 309, 300, 277, ($AlCl_3$ + HCl) 398, 313, 300, 275, (NaOAc) 376, 317, 274, (NaOAc + H_3BO_3) 377, 315, 264 ^{1}H NMR (400 MHz, DMSO-d_6) δ 7 64 (1 H, *dd*, *J* = 11 and 2 Hz, H-6′), 7 62 (1H, *d*, *J* = 2 Hz, H-2′), 7 53 (2 H, *d*, *J* = 8 5 Hz, H-2 coum H-6 coum), 7 52 (1 H, *d*, *J* = 16 Hz H-7 coum), 6 88 (1 H, *d*, *J* = 8 5 Hz, H-5′), 6 82 (2 H, *d*, *J* = 8 5 Hz,

Table 2 ^{13}C NMR data for flavonol glycosides*

C	Compound 1	2	3	4	5	6
2	156.5[a]	156.4[a]	156.4[a]	156 5[a]	157 2	157 1
3	133.3	133.0	133.1	132 9	133 9	133 6
4	177 5	177 4	177 4	177.4	177 7	177 6
5	161 4	161.2	161 2	161.2	161 0	160 9
6	98 8	98.7	98 7	98 8	99.5	99 4
7	164 3	164.1	164 2	164 4	163 0	162.9
8	93 7	93 6	93.7	93 8	94.7	94 6
9	156 6[a]	156 7[a]	156 6[a]	156 8[a]	156 1	156 0
10	104 0	103 9	103 9	103.9	105 7	105 6
1′	121.3	121 1	120 9	120 9	121.1	120 7
2′	115 3	115 2	130 9	131.0	115 4	131 1
3′	145 0[b]	144 9	115 0	115 0	145 0	115 1
4′	148.8	148.5	160 1	160.1	148 9	160 2
5′	116.5	116.3	115.0	115 0	116 4	115 1
6′	121 8	121 7	130 9	131 0	122 1	131 1
1″	101 5	101 2	101 5	101.4	102 0	101 8
2″	71 8	71.7	71 7	71.7	71 3	71 1
3″	71.2	71 0	70.9	71 0	73.2[e]	73 0[e]
4″	70.0	69 8	69 9	69.9	68 3	68.0
5″	71.6	71 4	71 5	71 5	73 8	73 6
6″	64.9	64.8	64 8	65.0	65 4	65 3
1‴	100 4	100 3	100.3	100 3	100.2	100 1
2‴	70.5[c]	70 4[c]	70.3[c]	70 4[c]	70 6[c]	70 4[c]
3‴	70.6[c]	70 5[c]	70 4[c]	70.5[c]	70 7[c]	70 6[c]
4‴	72 0	71 7	71.8	71 7	72.1	71 9
5‴	68 5	68.4	68 3	68.5	68 4	68 3
6‴	17.9	17.7	17.7	17 7	18 0	17 9
1⁗					100.0	99 9
2⁗					73 3[e]	73 1[e]
3⁗					77.3	77 2
4⁗					69.7	69 6
5⁗					76.5	76.4
6⁗					60 8	60.6
1⁗′	125 3	125 5	125 1	125.4		
2⁗′	130 4	133 0	130 2	132.9		
3⁗′	116.0	114 9	115 8	114.9[d]		
4⁗′	159 9	158 8	159 8	158 9		
5⁗′	116.0	114 9	115 8	114.9[d]		
6⁗′	130.4	133.0	130 2	132.9		
7⁗′	144.9[b]	143.8	144.7	143 7		
8⁗′	114.4	115 0	114.2	115 0[d]		
9⁗′	166 1	165 1	165 8	165.2		

*^{13}C NMR (100 MHz, DMSO-d_6)

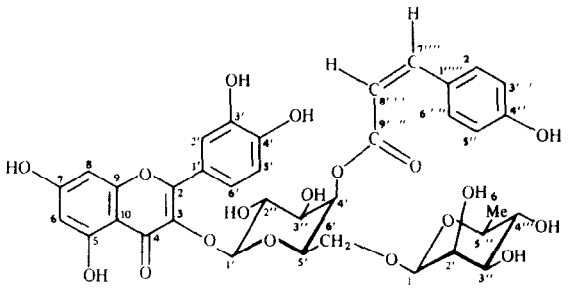
[a–e], Values marked with the same superscript within spectrum are interchangeable.

H-3 coum H-5 coum), 6 4 (1 H, *d*, *J* = 2 Hz, H-8), 6.39 (1 H, *d*, *J* = 16 Hz, H-8 coum), 6 2 (1 H, *d*, J = 2 Hz, H-6), 5 46 (1H, *d*, *J* = 7 5 Hz, H-1 gal), 4 38 (1H, *s*, H-1 rha), 3.3 (m, sugar protons), 0 93 (3 H, *d*, *J* = 6 Hz, Me-rha).

Quercetin 3-rhamnosyl (1→6)(4″-cis-p-*coumaroyl*)*galactoside* (variabiloside F) UV λ_{max}^{MeOH} nm. 313, 267 sh, 258, (NaOMe), 368, 271, ($AlCl_3$), 433, 306, 275, ($AlCl_3$ + HCl) 396, 303, 273; (NaOAc) 380, 318, 273, (NaOAc + H_3BO_3), 375, 313, 264. ^{1}H NMR (400 MHz, DMSO-d_6)· δ 7 75 (2 H, *d*, *J* = 8.5 Hz, H-2 coum H-6 coum), 7 63 (1 H, *dd*, *J* = 8 and 2 Hz, H-6′), 7 56 (1 H, *d*, *J* = 2 Hz, H-2′), 6 91 (1 H, *d*, *J* = 13 Hz, H-7 coum), 6.85 (1 H, *d*, *J* = 8 5 Hz, H-5′), 6 75 (2 H *d*, *J* = 8 5 Hz, H-3 coum H-5 coum), 6 4 (1 H, *d*, *J* = 2 Hz, H-8), 6 2 (1 H, *d*, *J* = 2 Hz, H-6), 5 9 (1 H, *d*, *J* = 13 Hz, H-8 coum), 5 48 (1 H, *d*, *J* = 7 5 Hz, H-1 gal), 4 4 (1 H, *s*, H-1 rha), 3 3 (*m*, sugar protons), 0 96 (3 H, *d*, *J* = 6 Hz, Me-rha)

Kaempferol 3-rhamnosyl (1→6) (4″-trans-p-*coumaroyl*) *galactoside* (variabiloside G) UV λ_{max}^{MeOH} nm 315, 267, (NaOMe) 372, 275, ($AlCl_3$) 396, 322 sh, 306, 276; ($AlCl_3$ + HCl) 394, 321 sh, 305, 277, (NaOAc) 376, 313, 275, (NaOAc + H_3BO_3), 317, 268 ^{1}H NMR (400 MHz, DMSO-d_6) δ8 1 (2H, *d*, *J* = 9 Hz, H-2′ H-6′), 7.53 (2H, *d*, *J* = 8 5 Hz, H-2 coum H-6 coum), 7 52 (1H, *d*, *J* = 16 Hz, H-7 coum), 6 9 (2H, *d*, *J* = 9 Hz, H-3′ H-5′), 6 8 (2H, *d*, *J* = 8 5 Hz, H-3 coum H-5 coum), 6 4 (1H, *d*, *J* = 2 Hz, H-8), 6 35 (1H, *d*, *J* = 16 Hz, H-8 coum), 6 2 (1H, *d*, *J* = 2 Hz, H-6), 5.44 (1H, *d*, *J* = 7 5 Hz, H-1 gal), 4 37 (1H, *s*, H-1 rha), 3 3 (*m*, sugar protons), 0 93 (3H, *d*, *J* = 6 Hz, Me-rha)

Kaempferol 3-rhamnosyl (1→6) (4″-cis-p-*coumaroyl*)



2

Fig. 1

galactoside (Variabiloside H) UV λ_{max}^{MeOH} nm 316, 266, (NaOMe) 378, 275, ($AlCl_3$) 394, 306, 275, ($AlCl_3$ + HCl) 391, 305, 276, (NaOAc) 381, 312, 275, (NaOAc + H_3BO_3) 315, 267. ^{1}H NMR (400 MHz, DMSO-d_6) δ8 05 (2 H, *d*, *J* = 9 Hz, H-2′ H-6′), 7 74 (2 H, *d*, *J* = 8 5 Hz, H-2 coum H-6 coum), 6 92 (1 H, *d*, *J* = 13 Hz, H-7 coum), 6 89 (2 H, *d*, *J* = 9 Hz, H-3′ H-5′), 6.75 (2 H, *d*, *J* = 8 5 Hz, H-3 coum H-5 coum), 6 42 (1 H, *d*, *J* = 2 Hz, H-8), 6 2 (1 H, *d*, *J* = 2 Hz, H-6), 5.81 (1 H, *d*, *J* = 13 Hz, H-8 coum), 5 46 (1 H, *d*, *J* = 7 5 Hz, H-1 gal), 4 38 (1 H, *s*, H-1 rha), 3 3 (*m*, sugar proton), 0 96 (3 H, *d*, *J* = 6 Hz, Me-rha)

Quercetin 3-rhamnosyl (1→6)galactoside-7-glucoside UV λ_{max}^{MeOH} nm 359, 266 sh, 256; (NaOMe) 404, 266, ($AlCl_3$) 440, 275, ($AlCl_3$ + HCl) 405, 364 sh, 270, (NaOAc) 416, 263, (NaOAc + H_3BO_3) 381, 261 ^{1}H NMR (400 MHz, DMSO-d_6) δ7 64 (1 H, *dd*, *J* = 8 5 Hz and 2 Hz, H-6′), 7 57 (1 H, *d*, *J* = 2 Hz, H-2′), 6.84 (1 H, *d*, *J* = 8 5 Hz, H-5′), 6 75 (1 H, *d*, *J* = 2 Hz, H-8), 6 44 (1 H, *d*, *J* = 2 Hz, H-6), 5 34 (1 H, *d*, *J* = 7 5 Hz, H-1 gal), 5 06 (1 H, *d*, *J* = 7 5 Hz, H-1 glc), 4 4 (1 H, *s*, H-1 rha), 3 3 (*m*, sugar proton), 1 05 (3 H, *d*, *J* = 6 Hz Me-rha)

Kaempferol 3-rhamnosyl (1→6)galactoside-7-glucoside UV λ_{max}^{MeOH} nm 350, 267, (NaOMe) 399, 268, ($AlCl_3$), 397, 354, 300, 275, ($AlCl_3$ + HCl) 396, 349, 300, 275, (NaOAc) 400, 266, (NaOAc + H_3BO_3) 353, 266 ^{1}H NMR (400 MHz, DMSO-d_6). δ 8 1 (2 H, *d*, *J* = 9 Hz, H-2′ H-6′) 6 87 (2 H, *d*, *J* = 9 Hz, H-3′ H-5′), 6 78 (1 H, *d*, *J* = 2 Hz, H-8), 6 45 (1 H, *d*, *J* = 2 Hz, H-6), 5 35 (1 H, *d*, *J* = 7 5 Hz, H-1 gal), 5 07 (1 H, *d*, *J* = 7 5 Hz, H-1 glc), 4 4 (1 H, *s*, H-1 rha), 3 3 (*m*, sugar protons), 1 06 (3 H, *d*, *J* = 6 Hz, Me-rha)

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