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OVIPOSITION STIMULANTS FOR THE MONARCH BUTTERFLY: FLAVONOL GLYCOSIDES FROM *ASCLEPIAS CURASSAVICA*

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Key Word Index—*Danaus plexippus*; Danainae; Nymphalidae: *Asclepias curassavica*; Asclepiadaceae; monarch butterfly; oviposition stimulants; quercetin glycosides.

Abstract—The monarch butterfly *Danaus plexippus* oviposits on milkweed plants, primarily within the Asclepiadaceae. Oviposition stimulants responsible for host plant recognition were isolated from *Asclepias curassavica*. Six flavonoid glycosides–quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside, quercetin 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-galactopyranoside, quercetin 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside, quercetin 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, quercetin 3-*O*-β-D-galactopyranoside, quercetin 3-*O*-β-D-glucopyranoside, and an unidentified flavonoid mixture were isolated and characterized from this plant. An additional glycoside, possibly quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-glucopyranoside, which could not be separated from the first triglycoside, was also found in some batches of plant extract. The two dirhamnosyl glycosides, the glucosylgalactose and the rutinoside were found to be active as oviposition stimulants at 0.5 g leaf equivalents.

INTRODUCTION

The monarch butterfly, *Danaus plexippus*, which belongs to the subfamily Danainae (Fam: Nymphalidae) generally uses the milkweeds as host plants. About 45 species mainly belonging to the Asclepiadaceae (40 spp.) have been reported as hosts [1]. Numerous studies have focused on host cardenolide content, palatability for larvae, and possible factors affecting the acceptance of these plants by the ovipositing butterflies [2, and refs cited therein]. Some authors have suggested that cardenolides are oviposition cues, but others have argued that cardenolides cannot explain host choice by these butterflies and that other compounds are likely to be involved. [3–5]. The cardenolide content of host plants varies considerably. In fact for some host plants like *Marsdenia* sp., cardenolides have not been reported. The chemistry of the Asclepiadaceae is highly diverse and various classes of compounds such as cardenolides, steroidal glycosides (mainly pregnane derivatives), phenanthroindolizidine alkaloids, steroidal alkaloids and a few flavonoids have been reported. However, the involvement of any of these chemicals in the monarch–milkweed associaiton has yet to be demonstrated.

In the current paper we report the isolation and identification of quercetin tri- and di-glycosides as contact oviposition stimulants for the monarch butterfly from *Asclepias curassavica*.

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RESULTS AND DISCUSSION

Isolation of active compounds

The isolation of oviposition stimulants was monitored by bioassays. The ethanolic extract of *A. curassavica*, applied to green sponges at 1 g leaf equivalent (1 gle), induced egg laying by monarch butterflies. The ethanolic extract was fractionated into hexane, chloroform, ethyl acetate and *n*-butanol layers by solvent extraction. The activity was primarily in the *n*-butanol extract, although some activity remained in the post-butanol aqueous fraction. The HPLC trace of *n*-butanol and post-butanol aqueous extracts indicated that some compounds were present in both extracts. Hence, after ethyl acetate extraction, the aqueous layer was exhaustively extracted with *n*-butanol, and the total *n*-butanol fraction was backwashed with water. As a result, only very minor activity remained in the post-butanol aqueous extract.

The *n*-butanol extract was further fractionated on a HPLC C_{18} reverse phase column (Bondex C18, Phenomenex) and monitored by a photo diode array (PDA) detector at 218 and 254 nm. Five fractions (A, B, C, D and E) were separated on the basis of HPLC pattern. Fractions A and B contained compounds that were also found in the post-butanol aqueous extract. Fractions C and D were mainly flavonoids, and two compounds were present in fraction E (detected by PDA).

The *n*-butanol extract was bioassayed for activity at different dosage levels (1.0, 0.5, 0.25, 0.1 and 0.05 gle) and was active at >0.05 gle (Table 1). Fractions C and

D were significantly active on their own or in combination. Fraction D gave a single spot on TLC, which was identified as rutin by comparison with an authentic sample. The HPLC trace of fraction C showed five peaks. For isolation of larger quantities of compounds in fraction C, the *n*-butanol fraction was initially separated by a C_{18} open column using a water–methanol gradient, and relevant fractions, containing the compounds detected in fraction C, were further separated into individual compounds by HPLC on a C_{18} column. Of the five peaks observed for fraction C, peaks **1–3** were derivatives of quercetin, according to their UV spectra. Peaks **4** and **5** were two very closely related compounds, which were not quercetin derivatives. Fractions D and E, as well as the ethyl acetate fraction, contained compounds **6–8**, which were identified as rutin, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside, respectively, by comparison of their spectral properties, TLC and HPLC with those of authentic samples. Compounds **1** and **2** were bioassayed and each was significantly active on its own. Fraction C was assayed against fraction C + B and against the total *n*-butanol fraction to test for possible synergism or deterrent effects. The results indicated that flavonoids alone could account for all the stimulatory activity (Table 1), and the combined effect of all the flavonoids was greater than that of the individual representatives.

Table 1. Oviposition responses of the monarch butterflies to *A. curassavica* fractions and compounds

Treatment	Control	*N*	*p*-value*
Butanol fraction	Solvent (water)	6	0.0207
C + D of butanol fraction	Solvent (water)	8	0.0540
C + D of butanol fraction	Butanol (fraction)	5	> 0.5
C of butanol fraction	Solvent (water)	10	0.0010
D (**6**) of butanol fraction	Solvent (water)	6	0.0469
C of butanol fraction	B + C of butanol fraction	6	> 0.5
Compound **1**	Solvent (water)	7	0.0089
Compound **2**	Solvent (water)	9	0.0488

*Wilcoxon's paired sign rank test was used to analyse the data, on the basis of eggs laid on the treatment and control sponges in paired choice bioassays.

Table 2. ^{1}H NMR of compounds **1a**, **1b**, **2** and **3** at 399.952 MHz

C–H	**1a***	**1a†**	**1b***	**1b†**	**2***	**2†**	**3†**
6	6.16	6.16 (*d*, 1.6)	6.16	6.16 (*d*, 1.6)	6.18	6.17 (*d*, 1.6)	6.15 (*d*, 2.0)
8	6.36	6.31 (*d*, 1.6)	6.36	6.31 (*d*, 1.6)	6.36	6.39 (*d*, 1.6)	6.33 (*d*, 2.0)
2′	7.50	7.55 (*d*, 2.0)	7.50	7.55 (*d*, 2.0)	7.52	7.62 (*dd*, 1.6, 8.4)	7.59 (*dd*, 2.4, 8.4)
5′	6.80	6.86 (*dd*, 8.3, 2.0)	6.80	6.86 (*dd*, 8.3, 2.0)	6.82	6.86 (*d*, 8.4)	6.85 (*d*, 8.4)
6′	7.72	7.66 (*d*, 8.3)	7.72	7.66 (*d*, 8.3)	7.65	7.92 (*d*, 1.9)	7.68 (*d*, 1.6)
1″	5.58	5.66 (*d*, 7.6)	5.54	5.56 (*d*, 7.6)	5.32	5.13 (*d*, 8.0)	5.73 (*d*, 7.6)
2″		3.80 (*m*)		3.64 (*m*)	3.54	3.58 (*dd*, 5.8, 8.0)	
3″					3.75		
4″					3.36		
5″					3.53		
6″							
1‴	5.10	5.21 (*d*, 1.2)	5.06	5.20 (*d*, 1.2)	4.06	4.13 (*d*, 7.6)	5.20 (*s*)
2‴		3.93 (*m*)			3.93 (*m*)	2.82	
3‴					2.96		
4‴					3.34		
5‴		3.50 (*m*)		3.39 (*m*)	3.40		
6‴		1.17 (*d*, 4.8)		1.07 (*d*, 5.2)			0.91 (*d*, 6.0)
1⁗	4.40	4.54 (*d*, 1.2)	4.32	4.49 (*d*, 0.8)			
5⁗		4.05 (*m*)		3.92 (*m*)			
6⁗		0.92 (*d*, 4.8)		0.97 (*d*, 5.2)			

*Solvent: DMSO-d_6.
†Solvent: CD_3OD.
J values are in Hz.

Identification of compounds **1**, **2** and **3**

Compound **1** was isolated as a single peak from the *n*-butanol extract by HPLC using the PDA detector. The UV absorption was typical of a flavonol which could be further characterized as a quercetin glycoside (see Experimental) UV shift reagent studies indicated it to be a 3-*O*-substituted quercetin derivative. Mass spectrometry (MS) gave an $[M^+ + Na]$ at *m/z* 779 indicating a M_r 756, which was consistent with a triglycoside. The ^1H NMR spectrum showed that it was a mixture of two compounds (**1a** and **1b**) with identical shifts for the aglycone moiety, but differing in the positions of anomeric protons and other sugar protons. The quantity of compound **1b** varied in different plant samples, and one sample provided almost pure compound **1a**. This sample was used for further identification.

Hydrolysis of **1a** yielded galactose and rhamnose, which were identified by comparing the paper chromatography (PC) and GC of TMSi derivatives with those of authentic samples. The GC indicated that the ratio of rhamnose to galactose was 2:1. The ^1H NMR of **1a** further confirmed the presence of two rhamnose (signals at δ1.17 and 0.9 for two methyl groups) and one galactose moieties, which accounted for the $[M^+]$ and a molecular formula of $C_{33}H_{40}O_{20}$.

Our conclusion that **1a** is a triglycoside of quercetin substituted at the 3 position was further confirmed by proton shifts of the aglycone moiety (Table 2). The anomeric proton shifts were observed at δ5.6, 5.1 and 4.5 for galactose, rhamnose 1‴ and rhamnose 1⁗, respectively. The downfield shift of the galactose and one of the rhamnose protons indicated that these lie in the deshielding region of the aglycone moiety, thus suggesting that the substitution of one of the rhamnoses is at the 2 position on the galactose. This was further confirmed by comparison with literature values for 2″ substituted sugars. On the basis of anomeric shift, the second rhamnose was considered to be at the 6 position and was attached to galactose. This was confirmed by ^{13}C NMR values for galactose (Table 3). The coupling constants of anomeric protons indicated that galactose and rhamnose have β-D-galactopyranose (J = 6 Hz) and α-L-rhamnopyranose (J = 1.2, 0.5 Hz) configurations, respectively. The data thus suggest that **1a** is quercetin 3-*O*-(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside. This compound, along with an isorhamnetin analogue, has been isolated from *Lysimachia fortunei* [6] and from *L. nummularia* (Primulaceae) [7]. It has also been isolated from *Alangium platanifolium* var. *trilobum* belonging to the Alangiaceae [8]. This is the first report of this compound from Asclepiadaceae. Kaempferol analogues have been reported from *L. mauritiana* and *Chenopodium quinoa* [9, 10]. Proton and partial ^{13}C NMR assignments for **1a** were based on ^1H–^1H COSY and ^{13}C–^1H correlation spectra. Values matched well with the known literature values.

Separation of **1b** from **1a** could not be achieved by the usual methods. Hence, further studies on **1b** were conducted on the mixture, with our prior knowledge of spectra for **1a**. The mixture of **1a** and **1b** on acid hydrolysis gave an additional sugar which was identified as glucose on the basis of its retention time on GC as the TMSi derivative and co-PC with an authentic sample. The ^1H NMR of the mixture gave additional signals for anomeric protons of sugars at δ5.56, 5.21 and 4.49 and CH_3 protons of rhamnose at 1.07 and 0.97 for 1‴ and 1⁗, respectively, for **1b**, while the aromatic protons did not show any difference. The ^{13}C NMR of the mixture in CD_3OD gave additional peaks at δ99.877 (C‴-1), 80.03 (C‴-2) and 68.367 (C‴-6) for glucose, whereas signals for rhamnoses could not be easily distinguished from signals for rhamnoses of **1a**. The coupling constants for anomeric protons suggested that galactose is in the β-pyranose form and rhamnoses are in the α-L-pyranose from (Table 2). These data suggested that **1b** is quercetin

Table 3. ^{13}C NMR of compounds **1a** and **1b** and related compounds from literature

C	1a	1b	1c	1d
2	156.3		156.5	156.4
3	132.7		132.9	133.0
4	177.0	176.1	177.5	177.3
5	161.1	161.3	161.3	161.3
6	98.9		98.9	98.8
7	165.1		164.0	164.0
8	93.6		93.7	93.6
9	155.9	155.9	156.5	156.4
10	103.5		104.2	104.2
1′	121.9		121.3	121.9
2′	115.1	115.9	130.6	115.3
3′	144.9	144.9	115.2	144.8
4′	148.5		156.5	148.2
5′	115.6		115.2	116.1
6′	121.0		130.6	121.6
1″	99.8	103.4	99.2	99.2
2″	74.7	80.0	75.6	75.7
3″	73.2		73.7	73.9
4″	68.5		68.3	68.8
5″	73.9		74.0	73.8
6″	64.9	68.0†	65.8	65.8
1‴	100.5	99.9	100.7	100.6
2‴	70.6†		70.9	70.9
3‴	704.†		70.8	70.5
4‴	71.7‡		72.3	72.2
5‴	68.3§	68.4†	68.8	68.9
6‴	17.2	17.0	17.2	17.2
1⁗	99.5	98.9	100.3	100.3
2⁗	70.6†		70.9	70.9
3⁗	70.4†		70.5	70.5
4⁗	71.8‡		72.3	72.2
5⁗	68.2§	68.2†	68.3	68.3
6⁗	17.9	17.5	17.7	17.7

*Solvent CD_3OD and all others in DMSO-d_6.

1c = Kaempferol 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside [9].

1d = Quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside [6].

†‡§Values exchangeable.

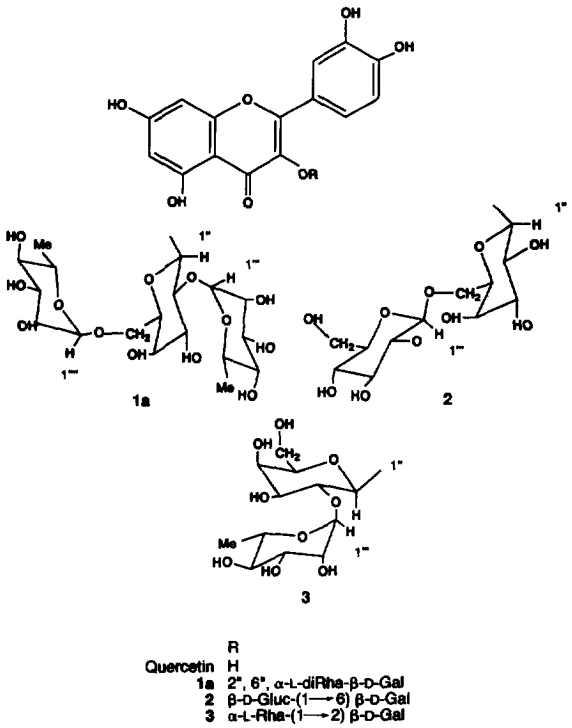
3-*O*-(2″,6″-*O*-di-α-L-rhamnopyranosyl)-β-D-glucopyranoside. This compound has been previously reported from several sources [11, 12].

Compound **2** was also shown to be a 3-*O*-substituted quercetin derivative on the basis of UV spectral studies. The MS showed $[M^+ + Na]$ at *m/z* 649, giving a M_r 626, and a molecular formula $C_{27}H_{30}O_{17}$, which indicated that it was a diglycoside. Hydrolysis of this compound yielded glucose and galactose. Comparison of spectral data with literature values confirmed the identity of **2** as quercetin 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-galactopyranoside, which has been reported from *L. fortunei*, *Solanum nigrum* and other sources [6, 13]. Partial proton assignments were based on ^1H–^1H COSY. Signals for H-6″ and H-6‴ could not be easily differentiated as they were complex and overlapped in the region δ3.4 to 3.7.

Compound **3** gave $[M^+ + Na]$ at *m/z* 633, M_r 610, giving a molecular formula $C_{27}H_{30}O_{16}$. Hydrolysis with acid gave galactose and rhamnose. Comparison of spectral data with literature data indicated that it was quercetin 3-*O*-α-(2″-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside [6, 9, 10, 13].

Although the chemistry of *Asclepias* has been investigated in great detail, the emphasis has been on cardenolides. Few reports are available on flavonoids, although Subramanian and Nair [14] found that Asclepiadaceae plants are rich in quercetin and kaempferol. Bate-Smith [15] detected kaempferol and quercetin in *Vincetoxicum officinale* and *Stephanotis floribunda*. Both *Vincetoxicum* and *Stephanotis* spp. are known to be hosts for the related *Danaus genutia* and *D. affinis*. Gibbs [16] reported quercetin 3-glucoside from seven species of *Asclepias*, but did not find quercetin and kaempferol in *Asclepias curassavica*. Hesperetin 7-*O*-rhamnoglucoside has been reported from *A. curassavica* [17]; kaempferol, quercetin, isorhamnetin and 3,5,3′4′-tetrahydroxy-7,8-(2″,2″-dimethyl-4″-methyl-5″,6″) pyranoflavone have been reported from *A. syriaca* [18]; and rutin, quercetin, nicotiflorin and kaempferol have been reported from *A. incarnata* [19]. Wyatt and Hunt [20] reported several mono-, di- and tri-glycosides of kaempferol and quercetin from four species of *Asclepias* and their hybrids, but their conclusions were based on the identification of hydrolysis products.

Flavonoids have been shown to be essential ingredients of contact stimulants for butterflies within the family Papilionidae. Luteolin 7-*O*-(6″-*O*-malonyl)-β-D-glucopyranoside has been identified as a component of the stimulants for umbellifer-feeding *Papilio polyxenes* along with chlorogenic acid and a cyclitol [21]. Vicenin-2, rutin, narirutin and hesperidin are oviposition stimulants for *P. xuthus* L. [22, 23] and hesperidin and naringin stimulate oviposition by *P. protenor* [24, 25]. Recently, Nishida reported a new flavonol triglycoside—isorhamnetin 3-*O*-glycosyl-(1 → 6)-galactoside-7-*O*-glucoside—as one of the essential components of the oviposition stimulant for *Luehdorfia japonica* from *Heterotropa aspera* [26]. For the *Papilio* spp., the flavonoids are active as contact stimulants only in the presence of other compounds such as chlorogenic acid, cyclitols and some



bases. In the case of *P. polyxenes*, plant volatiles also play a role in oviposition by increasing the frequency of landing on the plants [27]. However, the involvement of host volatiles in recognition of milkweed by monarch butterflies has not yet been demonstrated. Whole plants were present in our bioassay cages to provide a more natural environment that would include the presence of volatiles.

Our report is the first of its nature, in which flavonoids are active on their own as contact oviposition stimulants. Other butterflies that oviposit in response to single compounds include *Junonia coenia* (Nymphalidae), which uses iridoid glycosides to recognize *Plantago lanceolata* [28], and *Pieris* spp. (Pieridae), which are stimulated by glucosinolates in crucifer plants [29]. The involvement of multicomponent systems for host recognition appears to be characteristic of the ancestral papilionid butterflies.

Flavonoids are important components of the stimulants for the tribe Papilionini, whereas aristolochic acids are used by the tribe Triodini [30]. The use of flavonoids alone by the Danainae to recognize their hosts may provide some additional clues to the evolution and higher classification of the Lepidoptera.

EXPERIMENTAL

GC was run on a capillary column DB-5 (30 m × 0.23 mm) in a HP5890 GC linked to a HP 5970 MSD. All ^1H NMR were recorded at 399.99 MHz and ^{13}C NMR at 25.59 MHz. Mass spectra were obtained on a triple quadruple instrument, pneumatically assisted electron spray, + ve ion mode by direct infusion in

CH_3CN at 4 $\mu l\,min^{-1}$ with ionization at atmospheric pressure.

Plants. Asclepias curassavica were grown from seeds (Thompson and Morgan) in a greenhouse using Cornell mix artificial soil and grown under a 16 hr light:8 hr dark photo period at 25 ± 2°. Individual plants were transplanted to 25 cm pots, pruned when necessary and reused.

Insects. Butterflies were reared in a greenhouse, from larvae obtained as 2nd or early 3rd instars from a continuous culture [31]. They were reared on *A. curassavica* plants (occasionally on *A. incarnata*). Pupation occurred in 1 m^3 screened wooden cages in the same greenhouse at RH >65%. Freshly eclosed butterflies were transferred to 1 m^3 cages, equipped with feeders containing 10% sucrose solns. After 6–8 days, butterflies were mated and ready to lay eggs in bioassays. Test butterflies were deprived of host plants until they were used for experiments. They were briefly exposed to the host plants before bioassays were started.

Bioassays. Artificial leaves were made from light green sponges which were cut into 45 × 85 × 5–6 mm blocks with a bandsaw and thoroughly washed with H_2O to remove all chemicals. Test extracts were dissolved in 3–3.5 ml H_2O to provide the required concns (generally 0.5 gle) and applied uniformly with a pipette. Control sponges were treated with an equal amount of solvent. These sponges were inserted vertically into 125 ml flasks filled with H_2O such that they were 3–4 cm into the H_2O, to maintain the necessary moisture for oviposition. One control and one experimental flask were placed on an inverted plastic basket in a 1 m^3 cage provided with sucrose feeders, and an intact *A. curassavica* plant was placed under the basket as source of host volatiles. Each experimental cage contained at least 4 male and 4 female butterflies. At the end of the test period (usually 6–10 hr) the eggs on both treated and control sponges were counted. Stimulatory activity was indicated when significantly more eggs were laid on treated sponges, based on statistical analysis using one-tailed Wilcoxon's paired rank (non-parametric) test.

Extraction and isolation. Fresh young terminal leaves of *A. curassavica* were extracted with 95% EtOH (4 ml/g). The ethanolic extract was evapd to almost dryness *in vacuo* and the resulting mixt. was partitioned successively between H_2O and hexane, $CHCl_3$, EtOAc and *n*-BuOH. The *n*-BuOH layer was sepd by HPLC for bioassays. To isolate compounds for identification, the *n*-BuOH layer was flash chromatographed on a reversed phase C18 column (Bakerbond octadecyl C18) using a H_2O–MeOH gradient system. Final purification was carried out by HPLC sepn.

Quercetin 3-O-(2″,6″-α-L-*dirhamnopyranosyl*)-β-D-*galactopyranoside* (**1a**). MS: [M^+ + Na] 779, [M^+ + H] 757; molecular formula $C_{33}H_{40}O_{20}$ UV λ_{max} nm: 255, 263sh, 295sh and 355 (MeOH); 397, 310sh, 269 (MeOH–NaOMe); 433.7, 380, 302, 273.4 (MeOH–$AlCl_3$); 401.3, 380, 300, 268 (MeOH–$AlCl_3$–HCl); 378.8, 310, 271.1 (MeOH–NaOAc); 370.4, 300, 259.1 (MeOH–NaOAc–H_3BO_3). ^{1}H NMR (399.99 MHz, DMSO-d_6): Table 2. ^{13}C NMR (25.51 MHz, DMSO-d_6): Table 3.

Acid hydrolysis of **1a**. Compound **1a** was hydrolysed with 2 M HCl for 4 hr at 80°. The reaction mixt. was extracted with EtOAc. The EtOAc gave an aglycone, which was confirmed as quercetin by co-TLC. The aq. fr. was evapd to dryness, the TMSi derivative was subjected to GC with TMSi derivatives of standard sugars, and on the basis of R_t values the hydrolysis products were identified as rhamnose and galactose. The aq. fr. was also co-chromatographed with standard sugars on paper using C_6H_6–pyridine–HOAc–H_2O (5:1:3:3) and *n*-BuOH–HOAc–H_2O (4:1:5) solvent systems, and aniline hydrogen phthalate was used to visualize the sugars.

Quercetin 3-O-β-D-*glucopyranosyl*-(1 → 6)-β-D-*galactopyranoside* (**2**). MS: [M^+ + Na] at 649, [M^+] 626; molecular formula $C_{27}H_{30}O_{17}$. UV λ_{max} nm: 255.7, 270sh, 300sh and 359.5 (MeOH); 401.7, 320sh, 269.7 (MeOH–NaOMe); 433.1, 332, 308sh, 273.2 (MeOH–$AlCl_3$); 401, 344sh, 304sh, 273.2 (MeOH–$AlCl_3$–HCl); 384, 320, 270.1 (MeOH–NaOAc); 378.1, 306sh, 261.3 (MeOH–NaOAc–H_3BO_3). ^{1}H NMR (399.99 MHz, CD_3OD and DMSO-d_6): Table 2.

Acid hydrolysis of **2**. Hydrolysis was carried out as for **1a**. Glucose and galactose were identified as the sugars attached to quercetin.

Quercetin 3-O-(2″-O-α-L-*rhamnopyranosyl*)-β-D-*galactopyranoside* (**3**). MS: [M^+ + Na] at 633, 610; molecular formula $C_{27}H_{30}O_{16}$. UV λ_{max} nm: 254.8, 267sh, 298sh and 355.7 (MeOH); 399, 328sh, 269.6 (MeOH–NaOMe); 432.1, 360sh, 273.6 (MeOH–$AlCl_3$); 432, 330sh, 273 (MeOH–$AlCl_3$–HCl); 387, 320sh, 271.5 (MeOH–NaOAc); 373.7, 302sh, 260 (MeOH–NaOAc–H_3BO_3). ^{1}H NMR (399.99 MHz, DMSO-d_6): Table 2.

Acid hydrolysis of compound **3**. Hydrolysis was carried out as for **1a**. Galactose and rhamnose were identified as the sugars.

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