



SAPONINS FROM *GLIRICIDIA SEPIUM*

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(Received in revised form 29 September 1997)

Key Word Index—*Gliricidia sepium*; leguminosae; hederagenin; saponin.

Abstract—Three new hederagenin-based acetylated saponins isolated from the fruits of *Gliricidia sepium*, were identified by chemical and spectroscopic methods as hederagenin-3-*O*-(4-*O*-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside, hederagenin-3-*O*-(3,4-di-*O*-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside and hederagenin-3-*O*-(3,4-di-*O*-acetyl- α -L-arabinopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Gliricidia sepium (Jacq.) Walp. is a fast growing multipurpose legume cultivated throughout the tropics. It is the subject of intensive research because of its potential to enhance the productivity and sustainability of agricultural systems [1], and grows on the East and West coast of Mexico, South America, South India and the Philippines [2]. This legume is a tree used in Mexico as shade for cocoa and coffee plantations and for this reason it is called “Madre cacao” (mother of cocoa). It is also used as a poison for rodents and in fact, the Latin name *Gliricidia* means “rodent poison” [2, 3]. It is used also as a hedge plant and the flowers are utilised as a food in some places in Mexico. In the Philippines *G. sepium* is one of the best species for reforestation of denuded or grassland areas, and the leaves have been used with corn as silage for ruminant feeds [2, 4].

In the course of the investigation of *G. sepium* it exhibited potential insecticidal activity [5], and the heartwood is moderately resistant to attack by marine organisms [6]. The leaves are a useful source of ethylene for advancing the ripening of some fruits [7] and several papers refer to this plant as a nitrogen-fixing tree [8–12].

The isolation of flavones and chalcones from the heartwood [13–16], coumarin, *O*-coumaric acid, melilotic acid, ceryl alcohol, kaempferol glycosides and some hydrocarbons from the leaves [17–20], quercetin glycoside from the flowers [21], canavanine from the seeds have previously been reported [2]. The present

paper describes the isolation and characterization of new saponins from the fruits of the title plant.

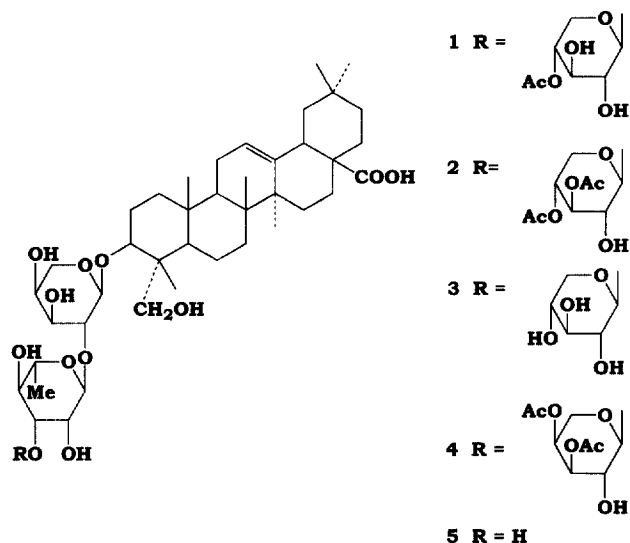
RESULTS AND DISCUSSION

When the chloroform extract of the fruits was treated in the usual manner, including reversed-phase and normal-phase column chromatography, three new saponins (**1**, **2**, **4**) and two known saponins (**3**, **5**) were isolated.

Compound **1** showed a $[M+Na]^+$ peak at m/z 947.4974 ($C_{48}H_{76}O_{17}+Na$). The presence in the 1H NMR and ^{13}C NMR spectra of six tertiary methyl signals, and eleven methylene signals all originating from the aglycone moiety indicated that the aglycone was an hederagenin-type triterpenoid. The presence of a trisubstituted olefinic linkage at C-12/C-13 was confirmed by the appropriate 1H NMR (δ 5.47) and ^{13}C NMR (δ 122.9, 145.0) signals. One primary hydroxyl group was assigned to the C-23 position based on the presence of hydroxy methylene signals at δ_H 3.93 and 4.28, δ_C 64.1, and one carbonyl carbon signal observed at δ_C 180.7 was assigned to C-28. The sugar moiety was assigned to the 3 β -position based on the presence of hydroxymethine signals at δ_H 4.29, δ_C 81.3.

On acidic hydrolysis, compound **1** provided arabinose, rhamnose and xylose (GC) as sugar moieties. In the 1H NMR and ^{13}C NMR spectra, three anomeric signals were observed at δ_H 5.03, δ_C 104.8 (arabinose), δ_H 6.34, δ_C 101.4 (rhamnose), δ_H 5.38, δ_C 107.2 (xylose). All protons of the three sugar units were assigned unambiguously from the COSY spectrum and a HMQC experiment correlated all the proton resonances with those of the corresponding carbons in each of the sugar units.

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Table 1. ^1H NMR spectral data for compounds **1**, **2** and **4** in pyridine- d_5

H	1	2	4
3	4.29*	4.29*	4.29*
12	5.47 (1H, <i>br s</i>)	5.48 (1H, <i>br s</i>)	5.47 (1H, <i>br s</i>)
23	3.93 (1H, <i>br s</i> , <i>J</i> 10 Hz)	3.92 (1H, <i>br s</i> , <i>J</i> 10 Hz)	3.92 (1H, <i>br s</i> , <i>J</i> 10 Hz)
	4.28*	4.30*	4.28*
24	1.14 (3H, <i>s</i>)	1.15 (3H, <i>s</i>)	1.14 (3H, <i>s</i>)
25	0.96 (3H, <i>s</i>)	0.97 (3H, <i>s</i>)	0.97 (3H, <i>s</i>)
26	1.03 (3H, <i>s</i>)	1.05 (3H, <i>s</i>)	1.05 (3H, <i>s</i>)
27	1.24 (3H, <i>s</i>)	1.25 (3H, <i>s</i>)	1.26 (3H, <i>s</i>)
29	0.93 (3H, <i>s</i>)	0.94 (3H, <i>s</i>)	0.94 (3H, <i>s</i>)
30	1.01 (3H, <i>s</i>)	1.01 (3H, <i>s</i>)	1.01 (3H, <i>s</i>)
Arabinose			
1	5.03 (1H, <i>d</i> , <i>J</i> 7 Hz)	5.04 (1H, <i>d</i> , <i>J</i> 7 Hz)	5.06 (1H, <i>d</i> , <i>J</i> 7 Hz)
2	4.58 (1H, <i>t</i> , <i>J</i> 7 Hz)	4.59 (1H, <i>dd</i> , <i>J</i> 7, 8 Hz)	4.59 (1H, <i>t</i> , <i>J</i> 7 Hz)
3	4.00 (1H, <i>dd</i> , <i>J</i> 4, 8 Hz)	4.01 (1H, <i>dd</i> , <i>J</i> 4, 8 Hz)	4.02 (1H, <i>dd</i> , <i>J</i> 4, 8 Hz)
4	4.13 (1H, <i>br s</i>)	4.13 (1H, <i>br s</i>)	4.13 (1H, <i>br s</i>)
5	3.67 (1H, <i>br d</i> , <i>J</i> 11 Hz)	3.67 (1H, <i>br d</i> , <i>J</i> 10 Hz)	3.67 (1H, <i>br d</i> , <i>J</i> 10 Hz)
	4.25*	4.26 (1H, <i>dd</i> , <i>J</i> 3, 12 Hz)	4.25*
Rhamnose			
1	6.34 (1H, <i>br s</i>)	6.36 (1H, <i>br s</i>)	6.35 (1H, <i>br s</i>)
2	4.88 (1H, <i>br s</i>)	4.90 (1H, <i>br s</i>)	4.91 (1H, <i>br s</i>)
3	4.76 (1H, <i>dd</i> , <i>J</i> 3, 9 Hz)	4.80 (1H, <i>dd</i> , <i>J</i> 3, 9 Hz)	4.82 (1H, <i>dd</i> , <i>J</i> 3, 9 Hz)
4	4.47 (1H, <i>t</i> , <i>J</i> 9 Hz)	4.44 (1H, <i>t</i> , <i>J</i> 9 Hz)	4.46 (1H, <i>t</i> , <i>J</i> 9 Hz)
5	4.72 (1H, <i>dd</i> , <i>J</i> 6, 9 Hz)	4.72 (1H, <i>dd</i> , <i>J</i> 6, 9 Hz)	4.72 (1H, <i>dd</i> , <i>J</i> 6, 9 Hz)
6	1.56 (3H, <i>d</i> , <i>J</i> 6 Hz)	1.55 (3H, <i>d</i> , <i>J</i> 6 Hz)	1.55 (3H, <i>d</i> , <i>J</i> 6 Hz)
Xylose			
1	5.38 (1H, <i>d</i> , <i>J</i> 7 Hz)	5.48 (1H, <i>d</i> , <i>J</i> 8 Hz)	
2	4.05 (1H, <i>t</i> , <i>J</i> 8 Hz)	4.09 (1H, <i>dd</i> , <i>J</i> 8, 9 Hz)	
3	4.22*	5.74 (1H, <i>t</i> , <i>J</i> 9 Hz)	
4	5.32 (1H, <i>dt</i> , <i>J</i> 6, 10 Hz)	5.27 (1H, <i>dt</i> , <i>J</i> 6, 10 Hz)	
5	3.52 (1H, <i>t</i> , <i>J</i> 11 Hz)	3.57 (1H, <i>t</i> , <i>J</i> 12 Hz)	
	4.19 (1H, <i>dd</i> , <i>J</i> 6, 11 Hz)	4.17 (1H, <i>dd</i> , <i>J</i> 6, 11 Hz)	
Arabinose (outer)			
1			5.46 (1H, <i>d</i> , <i>J</i> 7 Hz)
2			4.48 (1H, <i>t</i> , <i>J</i> 7 Hz)
3			5.54 (1H, <i>dd</i> , <i>J</i> 4, 10 Hz)
4			5.61 (1H, <i>br s</i>)
5			4.07 (1H, <i>dd</i> , <i>J</i> 2, 12 Hz)

* Overlapped with other signals.

The information concerning the sequence of the oligosaccharide chains and the linkage sites to the aglycone were obtained from the HMBC spectrum, the correlation peaks were observed from the following pairs: $H_{A-1}/C-3$, $H-3/C_{A-1}$, H_{R-1}/C_{A-2} , H_{A-2}/C_{R-1} , H_{X-1}/C_{R-3} , H_{R-3}/C_{X-1} , H_{X-4}/C_{Ac} . Therefore, the structure of **1** was elucidated to be hederagenin-3-*O*-(4-*O*-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Compound **2** showed a $[M+Na]^+$ peak at m/z 989.5154 ($C_{50}H_{78}O_{18}+Na$), and gave rise to 1H NMR and ^{13}C NMR spectra that were similar to those of **1**, except for the presence of signals due to one more acetyl residue. A cross-peak between the H_{X-3} and acetyl carbonyl carbon in the HMBC spectrum suggested that the acetyl residue was linked to the C-3 and C-4 positions of xylose. When the ^{13}C NMR data of **2** was compared with that of **1**, acylation shifts were observed at C_{X-3} . Therefore, compound **2** was characterized to be hederagenin-3-*O*-(3,4-di-*O*-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Compound **3** gave rise to 1H NMR and ^{13}C NMR spectra that showed the absence of the acetyl signal. Compound **3** was identified as hederagenin-3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside by comparison with an authentic sample from the roots of *Clematis chinensis* [22].

Compound **4** showed a $[M+Na]^+$ peak at m/z 989.5085 ($C_{50}H_{78}O_{18}+Na$). On acidic hydrolysis hederagenin, two arabinose, one rhamnose and two acetyl residues were produced. The 1H NMR and ^{13}C NMR spectra of **4** also indicates the presence of these moieties. In the HMBC spectrum, the correlation peaks were observed from the following pairs: $H_{A-1}/C-3$, $H-3/C_{A-1}$, H_{R-1}/C_{A-2} , H_{A-2}/C_{R-1} , $H_{A-1}/C-3$, H_{R-3}/C_{A-1} , H_{A-3}/C_{Ac} , H_{A-4}/C_{Ac} . Therefore, the structure of **4** was elucidated to be hederagenin-3-*O*-(3,4-di-*O*-acetyl- α -L-arabinopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside. The acetyl free compound of **4** has been obtained from the stems of *Clematis montana* [23].

Compound **5** gave rise to 1H NMR and ^{13}C NMR spectra that showed the absence of the third (outer) sugar. Compound **5** was identified as hederagenin-3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside by comparison with an authentic sample from *Hedera rhombea* [24].

EXPERIMENTAL

General

NMR spectra were recorded on a JEOL JNM-A500 spectrometer in pyridine- d_5 with TMS as int. standard. FAB-MS were recorded on a JEOL JMS-DX300 spectrometer. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. GC was run on a Shimadzu GC-6A gas chromatograph (column: 3 mm $\times$ 2 m, N_2 at 70 ml min $^{-1}$, 3% SE-30; temp. 170 $^\circ$).

Plant material

The fruits of *Gliricidia sepium* were collected from Mexico. It was identified by Dr Guillermo Suarez Ortega in the Jardin de Etno-botanico, Instituto

Table 2. ^{13}C NMR spectral data for compounds **1**, **2** and **4** in pyridine- d_5

C	1	2	4
1	39.0	39.1	39.1
2	26.4	26.4	26.4
3	81.3	81.3	81.2
4	43.7	43.7	43.6
5	47.8	47.8	47.8
6	18.2	18.2	18.2
7	32.9	32.9	32.9
8	39.8	39.8	39.8
9	48.2	48.2	48.2
10	36.9	36.9	36.9
11	23.8	23.7	23.7
12	122.9	122.5	122.5
13	145.0	144.9	145.0
14	42.2	42.2	42.2
15	28.4	28.4	28.4
16	23.8	23.9	23.9
17	46.7	46.7	46.7
18	42.1	42.0	42.1
19	46.5	46.5	46.5
20	31.0	31.0	31.0
21	34.3	34.3	34.3
22	33.3	33.3	33.3
23	64.1	64.0	64.1
24	14.2	14.2	14.2
25	16.1	16.1	16.1
26	17.5	17.5	17.5
27	26.2	26.2	26.2
28	180.7	180.4	180.7
29	33.3	33.3	33.3
30	23.8	23.8	23.8
Arabinose			
1	104.8	104.8	104.7
2	75.2	75.2	75.2
3	75.2	75.3	75.2
4	69.7	69.8	69.7
5	66.3	66.4	66.3
Rhamnose			
1	101.4	101.4	101.4
2	72.0	72.0	72.0
3	82.7	82.1	82.1
4	73.1	72.9	73.1
5	69.6	69.7	69.8
6	18.4	18.4	18.4
Xylose			
1	107.2	106.8	
2	75.8	73.1	
3	74.8	75.6	
4	72.9	70.4	
5	63.4	63.0	
Arabinose (outer)			
1			106.9
2			70.2
3			74.0
4			69.3
5			64.3

Nacional de Antropologia e Historia, Mongolia, where a voucher specimen of the plant is deposited.

Extraction and isolation

The air-dried fruits of *G. sepium* (500 g) were extracted with CHCl_3 . After evapn of the CHCl_3 , a part of this mixture (5 g) was subjected to repeated CC on silica gel with CHCl_3 -MeOH (15:1-1:1). The crude saponin fraction was subjected to RP-18 Lobar CC (45% CH_3CN) to give **1** (6.5 mg), **2** (10.2 mg), **3** (7.8 mg), **4** (10.8 mg) and **5** (5.3 mg).

Acid hydrolysis

A sample of each compound (ca 1 mg) was hydrolysed with 2N TFA at 120° for 5 h. The reaction mixture was concentrated to yield a residue, which was trimethylsilylated. The trimethylsilyl derivative was examined by GC, which showed the presence of arabinose (R_f 12.8, 14.5) and rhamnose (R_f 13.6, 18.3) and xylose (R_f 19.0, 24.5) in the ratio 1:1:1 (**1-3**), 2:1:0 (**4**), 1:1:0 (**5**).

Hederagenin-3-O-(4-O-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside (1). A solid, $[\alpha]_D^{25} + 35.8$ (MeOH; c 1.0). FAB-MS m/z : 947.4974, $\text{C}_{48}\text{H}_{76}\text{O}_{17} + \text{Na}$ requires 947.4980.

Hederagenin-3-O-(3,4-di-O-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside (2). A solid, $[\alpha]_D^{25} + 124.1$ (MeOH; c 1.0), FAB-MS m/z : 989.5154, $\text{C}_{50}\text{H}_{78}\text{O}_{18} + \text{Na}$ requires 989.5085.

Hederagenin-3-O-(3,4-di-O-acetyl- α -L-arabinopyranosyl)-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside (4). A solid, $[\alpha]_D^{25} - 75.2$ (MeOH; c 1.0), FAB-MS m/z : 989.5085, $\text{C}_{50}\text{H}_{78}\text{O}_{18} + \text{Na}$ requires 989.5085.

Acknowledgements—The Plant material was collected with the aid of Mitsui Pharmaceuticals, Inc. Our thanks also to Miss S. Kato and Miss K. Takahashi for NMR and MS measurements.

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