

New Antimycobacterial Saponin from *Colubrina retusa*

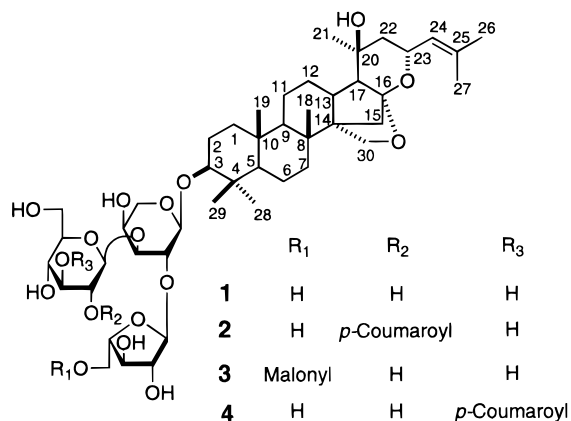
H. N. ElSohly,*† S. Danner,†§ X.-C. Li,† A. C. Nimrod,† and A. M. Clark†‡

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy, Thad Cochran Research Center, The University of Mississippi, University, Mississippi 38677

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A new jujubogenin saponin was isolated from the stems of *Colubrina retusa* and identified as jujubogenin 3-*O*- α -L-arabinofuranosyl (1 \rightarrow 2)-[3-*O*-(*trans*)-*p*-coumaroyl- β -D-glucopyranosyl (1 \rightarrow 3)]- α -L-arabinopyranoside (**4**) on the basis of chemical and spectroscopic data. The antimycobacterial activity expressed as minimum inhibitory concentration (MIC) for compound **4** was 10 μ g/mL.

Colubrina retusa (Ditter) Cowan is a dicotyledonaceous plant of the Rhamnaceae family, whose 55 genera and 900 species are widely distributed especially in the tropics. The only rhamnaceous plants of some economic importance are *Ziziphus* species and *Rhamnus purshiana*.¹ A previous study carried out on the stem of *C. retusa*² resulted in the isolation of a known saponin identified as jujubogenin [3-*O*- α -L-arabinofuranosyl (1 \rightarrow 2)- β -D-glucopyranosyl (1 \rightarrow 3)]- α -L-arabinopyranoside (**1**),³ along with two new saponins: jujubogenin 3-*O*- α -L-arabinofuranosyl (1 \rightarrow 2)-[2-*O*-(*trans*, *cis*)-*p*-coumaroyl- β -D-glucopyranosyl (1 \rightarrow 3)]- α -L-arabinopyranoside (**2**) and jujubogenin 3-*O*-(5-malonyl)- α -L-arabinofuranosyl (1 \rightarrow 2)-[β -D-glucopyranosyl (1 \rightarrow 3)]- α -L-arabinopyranoside (**3**). To isolate new constituents from this plant, further investigation of a column fraction yielded a new saponin (**4**) whose structure was established by spectroscopic means. ESI-MS showed a molecular ion peak at m/z 1067 [M + Na]⁺ suggesting the molecular formula C₅₅H₈₀O₁₉.



Comparison of the ¹H and ¹³C NMR spectra of **4** with the previously identified saponins **1** and **2** suggested the same type of jujubogenin aglycone. The ¹³C NMR of **4** displayed three anomeric carbon signals at δ 105.4, 104.8, and 110.4 as well as a typical carboxylic ester carbon signal at δ 167.5 along with some aromatic and olefinic carbon signals. Aromatic and olefinic proton signals were also observed in the downfield region of the ¹H NMR spectrum. On alkaline hydrolysis, compound **4** afforded **1** and *p*-coumaric acid. The

Table 1. ¹³C and ¹H NMR Data for Compound **4** in Pyridine-*d*₅ (ppm)

	δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)
aglycone-1	39.2	1.54, 0.75	Ara (<i>p</i>)-1	105.4	4.75 (d, 7)
2	26.8	2.10, 1.79	2	76.9	4.45
3	88.9	3.21 (dd, 11.7, 4.3)	3	83.6	4.16
4	40.0	—	4	68.3	4.50
5	56.7	0.66	5	65.4	4.15, 3.65
6	18.4	1.45, 1.33	Glc(<i>p</i>)-1	104.8	5.14 (d, 7.8)
7	36.4	1.47	2	73.4	4.02
8	37.8	—	3	79.3	5.92
9	53.6	0.86	4	70.0	4.32
10	37.6	—	5	78.5	3.94
11	22.0	1.51	6	62.6	4.43, 4.22
12	28.7	1.91, 1.88	Ara (<i>f</i>)-1	110.4	6.03 (d, 2.8)
13	37.5	2.79	2	83.4	4.97
14	53.9	—	3	78.3	4.78
15	37.0	2.42/1.49 (ABq, 8)	4	85.4	4.71
16	110.6	—	5	62.6	4.30, 4.14
17	54.6	1.35	<i>p</i> -Coum-1	126.5	—
18	19.0	1.04 (s)	2,6	130.5	7.43 (d, 8.6)
19	16.4	0.68 (s)	3,5	116.9	7.10 (d, 8.6)
20	68.7	—	4	161.3	—
21	30.1	1.33 (s)	α -	115.9	6.49 (d, 15.9)
22	45.6	1.74	β -	145.1	7.85 (d, 15.8)
23	68.8	5.12	O=C	167.6	—
24	127.2	5.48			
25	134.2	—			
26	25.5	1.66 (s)			
27	18.6	1.71 (s)			
28	28.2	1.24 (s)			
29	16.7	1.02 (s)			
30	65.9	4.23			
20-OH		5.93 (s)			

presence of *p*-coumaroyl group was indicated by the carbon signals at δ 115.9 (d), 116.9 (d), 126.5 (s), 130.5 (d), 145.1 (d), 161.3 (s), and 167.5 (s). The ¹H NMR spectrum showed the aromatic signals as two ortho-coupled doublets at δ 7.43 (2H, J = 8.6 Hz) and 7.10 (2H, J = 8.6 Hz) together with another two doublets at δ 6.49 (1H, J = 15.9 Hz) and 7.85 (1H, J = 15.9 Hz) integrating for one proton each; these were assigned to the olefinic protons, and the size of the coupling constant indicated a *trans* configuration. Complete assignments of the ¹H and ¹³C NMR signals were accomplished by 2D NMR including COSY, HMQC, and HMBC (Table 1). The linkage position of the *p*-coumaroyl residue to the sugar moiety was determined by 2D NMR. ¹H–¹H COSY and HMQC established each sugar's spin-coupling network. The signal of H-3 of glucose in **4** was significantly shifted to a downfield value of δ 5.92 when compared with **1**, in which it resonated at δ 4.22.² This indicated that the *p*-coumaroyl group was linked at the 3 position of glucose. Key HMBC correlations are sum-

* To whom correspondence should be addressed. Tel.: (601) 232-7610.

Fax: (601) 232-1006. E-mail: helsohly@olemiss.edu.

† National Center for Natural Products Research.

‡ Department of Pharmacognosy.

§ Current address: University of Vienna, Vienna, Austria.

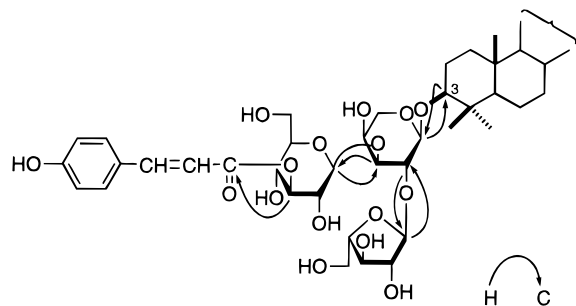


Figure 1. Key HMBC correlations of **4**.

marized in Figure 1. On the basis of the evidence above, compound **4** was established to be jujubogenin 3-*O*- α -L-arabinofuranosyl (1 \rightarrow 2)-[3-*O*-(*trans*)-*p*-coumaroyl- β -D-glucopyranosyl (1 \rightarrow 3)]- α -L-arabinopyranoside. Compounds **2** and **4** were active against *Mycobacterium intracellulare* (MIC of 50 and 10 μ g/mL, respectively); while **1** was inactive.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR were recorded in pyridine-*d*₅ with TMS as internal standard, using Bruker Avance DPX-300 (300 MHz) DRX-400 (400 MHz), and DRX-500 (500 MHz) instruments. ESI-FTMS spectra were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer. HPLC was performed on a Waters LC Module 1, using a Phenomenex Prodigy reversed-phase (150 \times 4.6 mm, 5 μ m) for screening and a Phenomenex Prodigy reversed-phase (250 \times 10 mm, 10 μ m) for preparative work; UV detection was achieved at 203 nm. Optical rotations were measured on a JASCO DIP 370 Digital Polarimeter.

Isolation. The stems from *C. retusa* L. (Rhamnaceae) were collected in Venezuela; a Voucher specimen (# V13021) was deposited at the National Center for Natural Products Research, University of Mississippi. Air-dried stems (500 g) were ground to a coarse powder and extracted at 37° with 95% EtOH (2.5 L \times 4, 3 h, each). The EtOH extract was suspended in water (1.5 L) and extracted with CHCl₃ (3 \times 1 L) and then with 1-BuOH (3 \times 1 L). The combined BuOH fractions were evaporated to dryness in-vacuo at 45° to afford a yellow residue (5.9 g). Part of the residue (5.0 g) was subjected to column chromatography on silica gel using a mobile phase consisting of CHCl₃/MeOH/H₂O mixtures starting at 70:10:1 and ending at 20:10:1 (6 L). Fractions of 25 mL each were collected, spotted on TLC, and combined according to TLC similarities. A total

of 17 combined fractions were obtained. The new saponin (**4**) was obtained from fraction #5 (109 mg) by further purification on HPLC using an isocratic mixture of 37% acetonitrile in water at a flow rate of 3.5 mL/min (*t*_R 19 min). This material was dissolved in 1.5 mL of MeOH, and 24 injections of 40–65 μ L each were made. The combined fractions were evaporated to dryness to yield compound **4**.

Jujubogenin 3-*O*- α -L-arabinofuranosyl (1 \rightarrow 2)-[3-*O*-(*trans*)-*p*-coumaroyl- β -D-glucopyranosyl(1 \rightarrow 3)]- α -L-arabinopyranoside (4**).** White powder (26.5 mg), mp 212 °C, [α]_D²⁵ -79.7° (c, 0.025, MeOH); ¹H and ¹³C NMR spectra are listed in Table 1; ESI-MS *m/z*, 1067.5199 [M + Na]⁺ (calcd for C₅₅H₈₀O₁₉Na, 1067.5186).

Alkaline Hydrolysis. A solution of **4** (1 mg) in 1% KOH (0.2 mL) was kept at room temperature for 30 min. The reaction mixture was subjected to TLC analysis using CHCl₃/MeOH/H₂O (30:10:1) as a developing system and 10% H₂SO₄ as a chromogenic reagent. Saponin **1** was detected with an *R*_f value of 0.72. For detection of the acid group, the reaction mixture was acidified with 2 N HCl, evaporated to dryness under a stream of N₂ and the residue dissolved in a drop of MeOH. The methanolic solution was spotted on TLC along with reference *p*-coumaric acid and the plate developed in CHCl₃/MeOH (6:1). *p*-Coumaric acid with an *R*_f value of 0.47 was detected by UV.

Antimycobacterial Assay. *Mycobacterium intracellulare* (ATCC#23068) was maintained on Lowenstein–Jensen slants at 4 °C. OADC-supplemented Middlebrook broth (5 mL) was inoculated with bacteria and incubated at 37 °C for 72 h. Compounds **1**, **2**, and **4** were solubilized in DMSO, further diluted in sterile saline and a 1:30 diluted inoculum of the bacteria in Middlebrook broth was added to the diluted samples in the wells of round-bottomed microtiter plates. The plates were incubated at 37 °C for 48 h and then visually assessed for growth. The antimycobacterial activity expressed as minimum inhibitory concentration (MIC) for compound **4** was 10 μ g/mL. Rifampicin was used as a positive control (MIC = 0.3 μ g/mL).

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References and Notes

- (1) Heywood, V. H., Ed. *Flowering Plants of the World*; Oxford University Press: 1993; pp 187–188.
- (2) Li, X.-C.; ElSohly, H. N.; Nimrod, A. C.; Clark, A. M. *J. Nat. Prod.* **1999**, *62*, 674–677.
- (3) Higuchi, R.; Kubota, S.; Komori, T.; Kawasaki, T.; Pandey, V. B.; Singh, J. P.; Shah, A. H. *Phytochemistry* **1984**, *23*, 2597–2600.

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