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A NEW MINOR HEDERAGENIN-TYPE TRITERPENE SAPONIN FROM *Cassia fistula*

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A new hederagenin-type triterpene saponin was found in the aqueous-ethanolic extract of the seeds of Cassia fistula *L. The structure of this compound was established as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-xylopyranosylhederagenin 28-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (**1**), mainly by extensive 2D NMR analysis in combination with spectral data of known compounds.*

Keywords: Fabaceae, *Cassia fistula* L., hederagenin-type, NMR, sapogenin.

Cassia fistula L., belonging to the *Cassia* genus, and thus the Fabaceae family, is a medicinal plant distributed in India and Southeast Asia [1]. In Vietnam, this plant is used not only for ornamental use because of its attractive yellow flowers but also for medicinal purposes. The fruits of *C. fistula* are currently used to treat throat disorders, inflammation, liver complications, chest problems, asthma, and rheumatism. Moreover, the seeds of this attractive plant are also used to treat gastritis, diarrhea, and biliousness, and to improve appetite [2]. In addition, it has been reported that different parts of *C. fistula* possess pharmacological properties such as anti-inflammatory, hepatoprotective, antidiabetic, antibacterial, antifungal, and antitumor activities [3–7]. Although previous studies on the seeds of *C. fistula* showed the presence of chemical substances that have been screened for potential pharmaceutical application, this portion has not been widely explored [8, 9]. The plant's wide occurrence and the saponin fraction's biological activities prompted us to conduct a detailed chemical investigation of the seeds of *C. fistula*. This paper reports their isolation and structure elucidation of a new minor hederagenin-type triterpene saponin.

Compound **1** was obtained as a white, amorphous powder, $[\alpha]_D^{25}$ –27° (*c* 0.75, MeOH). The molecular formula $C_{52}H_{84}O_{21}$ was estimated by the positive-ion HR-ESI-MS, which showed a $[M + Na]^+$ ion peak at *m/z* 1067.5409. The ^{13}C NMR spectrum of **1** displayed signal for six tertiary methyl groups at δ_C 15.1 (C-24), 17.7 (C-25), 18.9 (C-26), 27.4 (C-27), 34.5 (C-29), 25.3 (C-30), a hydroxymethyl carbon at δ_C 65.6 (C-23), an oxygen-bearing methine carbon at δ_C 83.6 (C-3), and an ester carbonyl group at δ_C 178.1 (C-28). The downfield C-3 signal at δ_C 83.6 and the upfield carbonyl signal at δ_C 178.1 of the aglycon suggested that compound **1** might be a bidesmosidic glycoside, with the sugar moieties attached at C-3 and C-28 of the aglycon [10, 11]. The ^1H NMR spectrum displayed the existence of six tertiary methyl groups at δ_C 0.84 (s, H_3-24), 0.97 (s, H_3-25), 1.07 (s, H_3-26), 1.18 (s, H_3-27), 0.81 (s, H_3-29), and 0.88 (s, H_3-30). These signals correlated with C-24, C-25, C-26, C-27, C-29, and C-30 at δ_C 15.1, 17.7, 18.9, 27.4, 34.5, and 25.3 in the HSQC spectrum. All the above evidence and the unsaturation between C-12 and C-13 were observed in the ^{13}C NMR spectrum at δ_C 124.2 and 145.6, respectively. The signal at δ_H 5.42 (br.t, J = 3.5 Hz, H-12) observed in the ^1H NMR spectrum confirmed the $\Delta^{12,13}$-hederagenin-type skeleton for the aglycon of **1**, which was in good agreement with the data given in the literature (Table 1) [12]. This was confirmed by comparing the signals of **1** with those of an oleanane-type triterpene glycoside isolated in the previous study. The structural differences between these compounds were located at H-3, H-4, H-5, and H-23 of the aglycone [13].

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TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** (Py-d_5, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	0.95, 1.54	40.2	27	1.18 (s)	27.4
2	1.94, 2.18	27.5	28	–	178.1
3	4.15 (dd, J = 11.1, 3.2)	83.6	29	0.81 (s)	34.5
4	–	44.7	30	0.88 (s)	25.3
5	1.61	48.9	Xyl I-1	4.96 (d, J = 7.6)	107.8
6	1.31, 1.65	19.7	2	4.15	77.5
7	1.63, 1.66	34.2	3	4.12	75.7
8	–	41.4	4	4.20	71.2
9	1.74	49.6	5	3.68, 4.25	68.1
10	–	38.5	Xyl II-1	4.78 (d, J = 7.6)	106.6
11	1.83, 1.97	25.3	2	3.92	75.9
12	5.42 (br.t, J = 3.5)	124.2	3	4.08	78.7
13	–	145.6	4	4.11	72.2
14	–	43.7	5	3.57, 4.26	68.3
15	1.46, 2.05	29.9	Rha-1	6.38 (br.s)	102.8
16	1.99, 2.10	24.9	2	4.75 (br.d, J = 3.1)	73.3
17	–	47.8	3	4.49 (dd, J = 9.1, 3.1)	73.6
18	3.11	43.4	4	4.29 (dd, J = 9.3, 9.1)	74.9
19	1.16, 1.72	47.8	5	4.46 (dq, J = 9.1, 6.4)	69.9
20	–	32.1	6	1.69 (d, J = 6.4)	19.8
21	1.12, 1.31	35.3	Glc-1	6.05 (d, J = 8.0)	96.0
22	1.76, 2.01	33.7	2	4.27	77.3
23	3.57, 4.18	65.6	3	4.20	80.3
24	0.84 (s)	15.1	4	4.25	71.8
25	0.97 (s)	17.7	5	4.02	78.8
26	1.07 (s)	18.9	6	4.22, 4.56	70.2

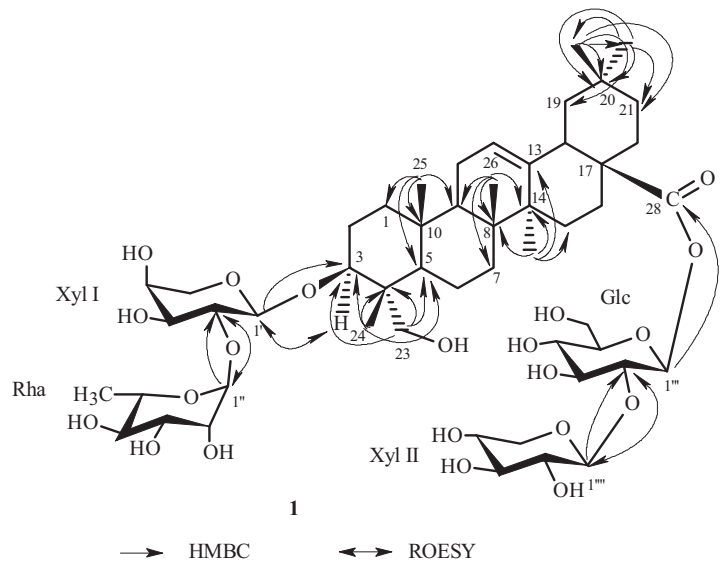

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Fig. 1. Key HMBC and ROESY correlations of compound **1**.

In the sugar region, the presence of four anomeric carbon signals was exhibited in the ^{13}C NMR spectrum at δ_C 107.8, 106.6, 102.8, and 96.0, which correlated with the protons at δ_H 4.96 (d, J = 7.6 Hz), 4.78 (d, J = 7.6 Hz), 6.38 (br.s), and 6.05 (d, J = 8.0 Hz) in the HSQC spectrum respectively, indicating the presence of four sugar moieties. Upon acid hydrolysis, compound **1** gave two xylose units, one rhamnose unit, and one glucose unit. The H_1 and H_2 coupling constants of 7.6 and 8.0 Hz indicate that the Xyl and Glc units occur as the β-anomers in $^{4}C_1$ configurations. The appearance of H-1 of the Rha unit

as a broad singlet showed that the Rha unit occurs as an α-anomer. A $J_{C\text{-}1,\ H\text{-}1}$ value of 167 Hz established the presence of the Rha unit as the α-anomer in the $^1C_4$ configuration [14, 15]. In the HMBC spectrum, the cross-peaks between δ_H 4.96 (d, J = 7.6 Hz, Xyl I H-1) and δ_C 83.6 (C-3 aglycon) and between δ_H 6.38 (br.s, Rha H-1) and δ_C 77.5 (Xyl I C-2) revealed the α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl sequence linked to the C-3 position of the aglycon. This identification was confirmed by the observation of the cross-peaks in the ROESY spectrum between δ_H 4.96 (d, J = 7.6 Hz, Xyl I H-1) and 4.15 (dd, J = 11.1, 3.2 Hz, Agl H-3), and between δ_H 6.38 (br.s, Rha H-1) and δ_H 4.15 (Xyl I H-2). The sequence and binding sites of the sugar units at the C-28 position of the aglycon were determined by the cross-peaks observed in the HMBC spectrum between δ_H 6.05 (d, J = 8.0 Hz, Glc H-1) and δ_C 178.1 (Agl C-28), and between δ_H 4.78 (d, J = 7.6 Hz, Xyl II H-1) and δ_C 77.3 (Glc C-2), together with the cross-peaks observed in the ROESY spectrum between δ_H 4.78 (d, J = 7.6 Hz, Xyl II H-1) and δ_H 4.27 (Glc H-2). Thus, the sugar sequence at C-28 of the aglycon was established as β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl.

According to the above results, the structure of **1** was characterized as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-xylopyranosylhederagenin 28-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (Fig. 1).

EXPERIMENTAL

General Experimental Procedures. Optical rotation values were recorded by an AA-10R automatic polarimeter (Optical Activity Ltd., Huntingdon, UK). The assignments of the NMR spectra were achieved based on the double resonance experiments measured on the NMR Inova 600 MHz spectrometer (Agilent Technologies, Santa Clara, CA, USA), including ^1H, ^{13}C, HSQC, HMBC, COSY, TOCSY, ROESY, and by the correlation with previously reported compounds in the literature data. The HR-ESI-MS spectrum was recorded on a Bruker micrOTOF II mass spectrometer (Bruker, Mannheim, Germany). Chemical shifts are given as δ-values with the reference to pyridine-d_5, the internal standard. Silica gel (RP-18, 75–200 μm; Silicycle, Quebec, Canada) was used for vacuum liquid chromatography (VLC). Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Merck, Germany) was used for column chromatography (CC). Silica gel 60 (15–40 μm; Merck, Germany) was used for medium-pressure liquid chromatography (MPLC). TLC was carried out on precoated 60 F254 (0.25 mm thick; Merck, Germany), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating.

Plant Material. The seeds of *C. fistula* L. were collected in a natural place in Thai Nguyen City, Vietnam, in 2023 (21°38′47.2″ N, 105°50′23.4″ E) and identified by one of the authors (Assoc. Prof. Thuy Thi Thu Vu, Ph.D.). A voucher specimen (No. CA.FI.LE.2301) was deposited in our laboratory.

Extraction and Isolation. Dried seeds (156.3 g) of *C. fistula* L. were pulverized and exhaustively extracted with EtOH–H_2O (75%–35%, three times, 500 mL each). The aqueous-ethanolic extract was combined and concentrated under reduced pressure to give a brown residue (15.2 g). A part of the extract (6.5 g) was separated by VLC over silica gel, elution being carried out using an EtOH–H_2O solvent system (0:1, 1:1, 0:1), to yield three fractions (NL.A, NL.B, and NL.C). Chromatography of the significant fraction NL.B (638.7 mg) on a MPLC over silica gel with $CHCl_3$–MeOH–H_2O (70:30:5, 60:32:7) resulted in seven subfractions (NL.B.1–NL.B.7). Subfractions NL.B.3 and NL.B.4 were combined (82.3 mg) and repeatedly subjected to a MPLC with the same procedure, yielding four subfractions (NL.B.1.a, NL.B.1.b, NL.B.1.c, and NL.B.1.d), with a few impurities. The saponin fraction NL.B.1.c (12.1 mg) was purified using CC on Sephadex LH-20, with EtOH 96%, as the eluent to provide compound **1** (3.3 mg).

3-*O*-β-D-Xylopyranosyl-(1→2)-β-D-xylopyranosylhederagenin 28-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (1), white, amorphous powder, $[\alpha]_D^{25}$ –27° (*c* 0.75, MeOH). ^1H NMR (600 MHz, Py-d_5) and ^{13}C NMR (150 MHz, Py-d_5) spectral data are shown in Table 1. HR-ESI-MS *m/z* 1067.5409 $[M + Na]^+$ (calcd for $C_{52}H_{84}NaO_{21}$, 1067.5397).

Acid Hydrolysis and GC Analysis. The protocol for the identification of sugar moieties and those absolute configurations of the isolated compounds was described in previous papers [15–17].

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