ISOLATION AND STRUCTURE ELUCIDATION OF THREE FLAVONOIDs FROM THE SEED OF ZIZIPHUS MAURITIANA LAM

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ABSTRACT

Three flavonoids, spinosin (1), 6′′′-feruloyl spinosin (2) and 6′′′-sinapoyl spinosin (3) were isolated from EtOH extract of seed of Ziziphus mauritiana. Their structures were elucidated by the analysis of their spectroscopic data (ESI-MS, 1H-NMR, 13C-NMR, DEPT, HSQC và HMBC) and compared with literatures. These compounds were isolated for the first time in Vietnam from the seed of Ziziphus mauritiana.

Keywords: Ziziphus mauritiana, spinosin, 6′′′-feruloyl spinosin and 6′′′-sinapoyl spinosin.

1. INTRODUCTION

The genus Ziziphus (Rhamnaceae), comprises of approximately 170 species and 12 variants, and is distributed in the warm-temperate and subtropical regions throughout the world. Ziziphus mauritiana Lam., an evergreen shrub up to 15 m height, is distributed in Vietnam. It is also widely growing throughout low-latitudes of Asia, Africa and Australia. In the Vietnamese Pharmacopoeia, the dry seeds of Z. mauritiana Lam have been used traditionally to tranquilize and relax the mind, soothing nerves (anxiolytic), and reducing sweating (anti-hydronic) effect [1]. Alkaloids [2, 6], flavonoids [2 - 6], triterpenoids [6] have been identified from the seeds of Z. mauritiana on the world. However, no chemical study was reported on the seeds of Z. mauritiana in Vietnam. The phytochemical investigation on the seeds of Z. mauritiana was carried on. This led to the isolation of Spinosin (1), 6′′′-feruloyl spinosin (2) và 6′′′-sinapoyl spinosin (3).

2. MATERIALS AND METHODS

2.1. General Experimental Procedures

1D and 2D NMR spectra were performed on Bruker AM 500 FT-NMR spectrometers with TMS as an internal standard. ESI-MS spectra were recorded on AGILENT 1200 series LC-MSD Ion Trap. TLC was performed on precoated TLC plates (DC-Alufoilien 60 F254 and RP18 F254
Merck) with compounds visualized by spraying the dried plates with 10 % aqueous H2SO4 followed by heating until the plate was dry or UV lamp (245 nm). Silica gel (240-430 mesh, Merck), reversed silica gel (ODS-60-14/63, Fujisilisa – Japan), diaion HP20SS (Supelco-USA) and Sephadex LH20 (Sigma) were used for column chromatography (CC).

2.2. Plant Material

The seeds of Z. mauritiana were collected from Phu Tho province and identified by Prof. Tran Huy Thai (The Institute of Ecology and Biological Resources). A voucher sample has been deposited in Hanoi University of Science and Technology.

2.3. Extraction and Isolation

The air-dried seeds (2 kg) of Z. mauritiana were powdered, and extracted three times per 3 hours with EtOH at 40 °C. The extracts (312 g) was suspended in H2O and degreased by n-hexan and ethyl acetate. The H2O-soluble layer was applied to a Diaion HP20SS column, eluting with H2O/MeOH (1:0→0:1), to give fractions W1–W5. Compound 1 (53 mg) was obtained by crystallization from fraction W2. Fraction W3 was subjected to Sephadex LH-20 chromatography column, eluting with MeOH to derive the subfractions W3a, W3b và W3c. Subfraction W3b was subjected to silica gel (EtOAc–MeOH, 1:0 → 0:1) to give W3b1 and W3b2. Subfraction W3b1 was further separated by reversed silica gel ODS-60 (MeOH) to provide compounds 2 (17 mg). Subfraction W3b2 was further separated by reversed silica gel ODS-60 (MeOH) to provide compounds 3 (12 mg).

Spinosin (1): A yellowish crystal with a molecular formula C28H32O15. ESI-MS m/z 609,01 [M+H]+. For 1H NMR (500 MHz DMSO-d6, room temperature) and 13C NMR (125 MHz, DMSO-d6) spectral data see Table 1.

6″′-feruloyl spinosin (2): A yellowish amorphous powder with a molecular formula C38H40O18. ESI-MS m/z 785,01 [M+H]+. For 1H NMR (500 MHz DMSO-d6, room temperature) and 13C NMR (125 MHz, DMSO-d6) spectral data see Table 2.

6″′-sinapoyl spinosin (3): A yellowish amorphous powder with a molecular formula C39H42O19. ESI-MS m/z 837.10 [M+Na]+. For 1H NMR (500 MHz DMSO-d6, room temperature) and 13C NMR (125 MHz, DMSO-d6) spectral data see Table 2.

3. RESULT AND DISCUSSION

Compound 1 was isolated as a yellowish crystal, Its molecular formula was deduced to be C28H32O15 on the basis of ESI-MS (m/z 609.01 [M+H]+). Its 1H and 13C-NMR spectra showed signals for a flavonoid glycoside [2].

In 1H-NMR spectrum, compound 1 exhibited a singlet at δH 13.62, 13.5 (1H, s, 5-OH) which was ascribed to 5-OH, substituted at carbon C-5 at δC 160.55/159.68. The 1H NMR spectrum of 1 indicated six aromatic proton signals due to aglycone moiety, i.e., a characteristic singlet signal at δ 6.83/ 6.85 due to the H-3 proton, a singlet at δ 6.77/6.80 (1H, s, H-8′) indicating A-ring with three substituents, an AA′BB′ aromatic proton system appearing at δ 7.98/7.96 (1H, d, J = 8.5 Hz, H-2′, 6′) and 6.95/6.93 (1H, d , J = 8.5 Hz, H-3′, 5′) indicating a C-4′ substituted B-ring, and a singlet at δ 3.89 (3H, s, 7-OCH3) due to methoxyl protons. In 13C-NMR spectrum, the carbon signal at δC 182.30/181.99 was assigned to the carbonyl carbon C-4.
The signals of two anomic protons at $\delta$ 4.70/4.68 (1H, d, $J = 9.5$ Hz, H-1′′) and 4.16 (1H, d, $J = 7.8$ Hz, H-1′′′) and 10 protons at $\delta$ 2.55-4.70 (4.70/4.68 (1H, d, $J = 9.5$, H-1′′), và $\delta$ 4.47/4.29 (1H, t, $J = 9.0$, H-2′′) và tạ các $\delta$ 3.44/4.43 (H-3′′), 3.16 (H-4′′), 3.18 (H-5′′) 3.70/3.68 (2H, H-6′′), 4.16 (1H, t, $J = 9.0$, H-1′′′), 2.86 (1H, m, H-3′′′), 2.98 (1H, H-4′′′), 2.55/2.75 (1H, m, H-5′′′), $\delta$ 3.16/2.98 (2H, b, H-6′′′)) together with the 13C NMR spectral data indicated the presence of two $\beta$-D-glucosyl moieties. The resonances for the carbons and protons of the aglycone and the sugar moiety had a close resemblance to those of spinosin, and they were assigned according to the $^1$H and $^{13}$C NMR spectral data for spinosin as well as its own HSQC spectrum.

The linkage between the anomic C-1′′ and C-6 and the interglucosidic linkage between C-2′′ and C-1′′′ were determined by the HMBC correlations of H-1′′ (δ 4.47/4.29)/ C-7 (δ 165.08/163.87) and C-5 (δ 160.55/159.68) and H-1′′′(δ 4.16)/C-2′′(δ 81.23/80.76) (figure 1 (1)). The chemical shift value of the anomic carbon C-1′′ and HMBC correlation between H-1′′ (δ 4.47/4.29) and C-6 (δ 108.53/108.58) indicated that the anomic carbon (C-1′′, δ 71.05/70.72) of $\beta$-glucose was connected to C-6 through a C-linkage.

**Table 1.** $^1$H-NMR and $^{13}$C-NMR spectra of spinosin.

| Pos. | $\delta_{ii}$ (J, Hz) | $\delta_{i}(J, \text{Hz})^*$ | $\delta_{ii}$ (J, Hz) | $\delta_{i}$ (J, Hz)$^*$ |
|------|----------------------|----------------------|----------------------|----------------------|
| 2    | 163.81/163.79, C     | 163.66/163.66        |
| 3    | 6.83/6.85 (s)        | 6.83/6.84            |
| 4    | 103.10/103.00, CH    | 102.90/102.99        |
| 5    | 182.30/181.99, C     | 181.82/182.15        |
| 6    | 160.55/159.68, C     | 159.57/160.42        |
| 7    | 108.53/108.58, C     | 108.58/108.58        |
| 8    | 165.08/163.87, C     | 163.72/164.94        |
| 9    | 6.77/6.80 (s)        | 6.67/6.80            |
| 10   | 90.78/90.31, CH      | 90.20/90.67          |
| 1′   | 157.09/156.97, C     | 156.85/156.96        |
| 2′   | 104.44/104.19, C     | 104.09/04.36         |
| 3′   | 121.08/121.04, C     | 120.92/120.92        |
| 4′   | 7.98/7.96 (d, 8.5)   | 7.97                 |
| 5′   | 128.55, C            | 128.34/128.34        |
| 6′   | 6.95/6.93 (d, 8.5)   | 6.95                 |
| 7-OMe| 116.00, C            | 115.59/115.89        |
| 1″   | 161.28, C            | 160.78/161.22        |
| 2″   | 3.89 (s)             | 3.92                 |
| 3″   | 56.54/56.12, CH      | 55.99/56.42          |
| 4″   | 4.70/4.68            | 4.67/4.69            |
| 5″   | 71.05/70.72, CH      | 70.64/70.99          |
| 6″   | 4.47/4.29            | 81.23/80.76          |
| 7″   | 78.68/78.29, CH      | 78.19/78.55          |
| 8″   | 3.44/3.43            | 3.16                 |
| 9″   | 70.46, CH            | 70.41/70.41          |
Thus, the spectral data of compound 1 were similar to Spinosin, previously reported in the literature [4]. The similar NMR phenomenon to that of spinosin, i.e. the appearance of serial separate signals, was observed. It is proposed that rotational isomers produced by the rotational barriers 7-OCH$_3$ in flavones-6-C-glycoside must exist in compound 1 [4].

![Figure 1. Structure and key HMBC correlations of compounds 1-3.](image)

Compound 2 was isolated as a yellowish amorphous powder. The molecular formula was determined to be C$_{38}$H$_{40}$O$_{18}$ from the [M+H]$^+$ ion peak at m/z 785.01 in the ESI-MS.

The resonances for the carbons and protons located on the flavonoid framework had a close resemblance to those of the spinosin based on the $^1$H and $^{13}$C NMR spectral data as well as its own HSQC spectrum.

The downfield shift of C-6‴ (δ 62.18 + 2.1 ppm) and the upfield shift of C-5‴ (δ73.45, + 2.8 ppm) relative to the corresponding signals of spinosin revealed the acylation of C-6‴, which is also supported by the corresponding signals of 6‴- feruloyl spinosin. The spinosin moiety accounted for a partial molecular formula of C$_{28}$H$_{31}$O$_{15}$. Thus, the remaining molecular formula should be C$_{10}$H$_{9}$O$_{3}$ as a substituent attached to the spinosin group at C-6‴.

The resonances for the carbons and protons of the substituent in the $^1$H and $^{13}$C NMR as well as HSQC spectra included an ABX aromatic proton system appearing at δ 6.78 (1H, d, $J=8.5$, H-5‴″), δ 6.93/6.79 (1H, dd, $J=8.0$, 2.0 Hz, H-6‴″), δ 7.17/7.04 (1H, d, $J=2.0$, H-2‴‴), a

| 5‴″ | 3.18 | 81.91/81.62, CH | 81.42/81.67 |
|-----|------|----------------|--------------|
| 6‴″ | 3.70/3.68(m) | 61.48, CH$_2$ | 61.40 |
| 1‴‴ | 4.16 | 4.15/4.17 | 103.43/105.25, CH | 105.08/105.22 |
| 2‴‴ | 2.83/2.85 | 74.73/74.56, CH | 74.48/74.62 |
| 3‴‴ | 2.86 (m) | 76.38/76.34, CH | 76.25/76.25 |
| 4‴‴ | 2.98 (m) | 69.47/69.18, CH | 69.23/69.52 |
| 5‴‴ | 2.55/2.75 (m) | 76.66/76.44, CH | 76.25/76.25 |
| 6‴‴ | 3.16/2.98 | 60.08/60.62, CH$_2$ | 60.10/60.59 |
| 5-OH | 13.62/13.5 | |

* Reference spectral data
methoxyl (δ 3.80 (3H, s, 3-OMe; δC 55.71/55.66), and a carbonyl (δC 166.42/166.32 (C-9’’’’)). The geometry of the trans C-7’’’’- C-8’’’’ double bond was confirmed on the basis of large coupling constant 15.5 Hz (7.22/7.80, d, J = 15.5, H-7’’’’ and 6.24/6.17, d, J = 15.5, H-8’’’’). The HMBC correlation between H-6’’’’/C-7’’’’, H-2’’’’/C-7’’’ as well as the proton of methoxyl and C-3’’’’ indicated the presence of a feruloyl moiety (figure. 1 (2)). Thus, the spectral data of compound 2 were similar to 6’’’-feruloylspinosin, previously reported in the literature [3, 4].

**Table 2.** $^1$H-NMR and $^{13}$C-NMR spectra of compounds 2 and 3.

| Pos. | $\delta_H$ (J, Hz) | $\delta_C$ | $\delta_H$ (J, Hz) | $\delta_C$ |
|------|-------------------|----------|-------------------|----------|
| 2    | 163.96/163.68     | 103.24   | 6.69/6.54(s)      | 103.91   |
| 3    | 6.85/6.71(s)      | 103.24   |                   | 103.91   |
| 4    | 182.33/181.88     | 181.75   |                   | 159.433  |
| 5    | 160.84/159.53     | 108.77   |                   | 108.67   |
| 6    |                   | 165.27/164.21 | 165.10    |
| 7    |                   | 90.68/89.98 | 90.45/89.86 |
| 8    |                   | 157.10/156.93 | 156.97    |
| 9    |                   | 104.51/104.03 | 103.91    |
| 1’   | 121.24            | 7.84/7.82(d,8.5) | 121.16    |
| 2’ 6’| 7.82/7.80(d,7.5)  | 128.64/128.53 | 7.84/7.82(d, 8.5) | 128.51/128/41 |
| 3’ 5’| 6.90/6.88(d,9)    | 116.09/115.88 | 6.91/6.86(d,8.5) | 115.75   |
| 4’   |                   | 161.23   |                   | 161.42   |
| 7-OMe| 3.90/3.87(s)      | 56.49/56.03 | 3.91/3.86(s)      | 56.36/56.07 |
| 1”   | 4.69/4.68(d, 9.5) | 71.06    | 4.70/4.69(d, 9.5) | 70.59    |
| 2”   | 4.48/4.26(m)      | 82.00/81.69 | 4.48/4.25(m)      | 81.937/81.63 |
| 3”   | 78.85/78.64       | 3.45(m)  | 78.78/78.53       |          |
| 4”   | 3.16(m)           | 70.31    | 3.17(m)           | 70.27    |
| 5”   | 3.16(m)           | 82.00    | 3.17(m)           | 81.63    |
| 6”   | 3.74(m)           | 61.54    | 3.82(m)           | 61.43    |
| 1””  | 4.28(d, 8)        | 105.81   | 4.29(d,8)         | 105.03   |
Compound 3 was isolated as a yellowish amorphous powder. The molecular formula was determined to be C_{39}H_{42}O_{18} from the [M+Na]^+ ion peak at m/z 837.10 in the ESI-MS.

All the protons and carbons of 3 appeared as pair signals in the 1H and 13C NMR spectra, which is characteristic of signals arising from a spinosin skeleton. In addition, a set of signals arising from one carbonyl (δC 166.27, C-9′′′′), two methoxyl [δC 55.59/55.54 (OCH₃ x 2)], four olefinic methines [δC 110.79/110.68 (CHx2), 147.85(CH), 113.59(CH)], and four aromatic quaternary carbons [δC 128.21(C), 149.19(CHx2), 140.45(C)] were observed in the 13C NMR (DEPT) spectra.

![Figure 2. Key HMBC correlations of sinapoyl moiety.](image-url)
Isolation and structure elucidation of tree flavonoids from the seed of *Ziziphus mauritiana* Lam

The $^1$H NMR spectrum displayed the existence of a symmetric 1,3,4,5-tetra-substituted aromatic ring [$\delta H 6.86/6.69$ (2H, s)], one trans double bond [$\delta H 7.24/7.10, 6.27/6.19$ (each 1H, d, $J = 15.5$ Hz, H-7'''', H-8''')] and two methoxy groups [$\delta H 3.81/3.79$ (6H, s, 3'''', 5''''-OMe)] and didn’t displayed the proton of 4'''. The aforementioned data suggested that 3 was a spinosin derivative acylated with a 3,5-dimethoxy,4-hydroxy-(E) cinamoyl (sinapoyl moiety). In the HMBC spectrum of 3, correlations between the H-6''' (\(\delta_H 4.05/3.57\)) and C-9''' (\(\delta_C 166.27\)) indicated the linkage of the sinapoyl moiety with C-6''' (\(\delta_C 62.01\)) of spinosin unit (figure 2, figure 1 (3)).

Thus, the spectral data of compound 3 were similar to 6'''-sinapoyl spinosin, compared with literature [6].

**4. CONCLUSION**

Three flavonoids, *spinosin* (1), 6'''- feruloyl spinosin (2) and 6'''-sinapoyl spinosin (3) were isolated from EtOH extract of seed of *Ziziphus mauritiana*. Their structures were elucidated by the analysis of their spectroscopic data (ESI-MS, $^1$H-NMR, $^{13}$C-NMR, DEPT, HSQC and HMBC) and compared with literatures. The $^1$H and $^{13}$C NMR spectra of 1, 2 and 3 showed the same phenomenon of separate signals because of rotational isomers which are produced by the rotational barriers 7-OCH3 in flavone-6-C-glycoside. It was proved that the multiplicity collapsed to a first-order spectrum when measuring at high temperature. Thus, structural elucidation of this type of compounds will be easy when measuring all spectra at high temperature. These compounds were isolated for the first time in Vietnam from the seed of *Ziziphus mauritiana*.

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Tóm tắt

Phân lập và xác định cấu trúc ba hợp chất flavonoid từ hạt táo ta

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Ba hợp chất spinosin (1), 6′′′- feruloyl spinosin (2) và 6′′′- sinapoyl spinosin (3) đã được phân lập từ cao chất phần đoạn nước của nhân hạt táo ta Ziziphus mauritiana Lam. Cấu trúc của chúng được xác định dựa trên số liệu phổ khối phân mzd từ ESI-MS và phổ cộng hưởng từ hạt nhân NMR (1H, 13C, DEPT, HSQC và HMBC) và so sánh với các tài liệu tham khảo. Các hợp chất này được phân lập lần đầu tiên ở Việt Nam từ hạt táo ta Ziziphus mauritiana Lam.

Từ khóa: táo ta, Ziziphus mauritiana, spinosin, 6′′′- feruloyl spinosin và 6′′′-sinapoyl spinosin.