Original Article

Chemical Consituents from *Abelmoschus Sagitifolius* (Kurz) Merr.

Dinh Ngoc Thuc1,*, Vu Thi Ha Mai1, Vu Thi Hue2, Bui Thu Ha3

1Hong Duc University, 565 Quang Trung, Dong Ve War, Thanh Hoa City, Vietnam
2Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
3Hanoi National University of Education, Cau Giay, Hanoi, Vietnam

Received 05 November 2021
Revised 14 April 2022; Accepted 25 April 2022

Abstract: *Abelmoschus sagittifolius* belongs to the *Abelmoschus* genus, Malvaceae family. Chemical study on the aerial parts of *Abelmoschus sagittifolius* collected in Vinh Hung commune, Vinh Loc district, Thanh Hoa province have led to the isolation of six compounds including sitostenone (1), friedelin (2), vomifoliol (3), vanilic acid (4), ketopinoresinol (5) and daucosterol (6). The structures of these compounds have been elucidated by NMR, MS spectroscopic data and comparison with the reported literatures. Compounds 1-2 and 4-5 were isolated for the first time from *Abelmoschus sagittifolius*.

Keywords: *Abelmoschus sagittifolius*, friedelin, vomifoliol, vanilic acid, ketopinoresinol, daucosterol.

1. Introduction

*Abelmoschus* is a genus of about fifteen species of flowering plants in the family of Malvaceae, native to tropical Africa, Asia and northern Australia. It was formerly included within *Hibiscus*, but is now classified as a distinct genus [1]. In traditional medicine, the root tuber of *A. sagittifolius* species is used as a health food and is used to treat diseases such as cough, constipation, neurasthenia, malnutrition, boils, back pain, dizziness and stomach pain.

The roots of *A. sagittifolius* are rich in mucilage and starch [2]. Qualitative results of the roots of *A. sagittifolius* in Bac Lieu showed phytosterols, coumarins, fatty acids, organic acids, amino acids, reducing sugars and uronic compounds [3]. A study about *A. sagittifolius* collected in Ha Trung district, Thanh Hoa province revealed 5 substances including ventricosin A, 4(15)-eudesmene-11-on, tagitin A, β-sitosterol and daucosterol.[4] From the roots of *A. sagittifolius* in Hainan island, Chinese scientists isolated several compounds including cadinane sesquiterpene, lignan and amide derivatives [5, 6]. Recently, we isolated 4 compounds: 5,12-epoxy-9-hydroxy-7-megastigmen-3-on, bombaxon, 3-O-[β-D-glucopyranosyl]bombaxon and 2β,7,3-
trihydroxycalamenen 3-O-β-D-glucoside from the roots of *A. sagittifolius*. In our continuing research of this plant, herein we described the isolation of six compounds including sitostenone (1), friedelin (2), vomifoliol (3), vanillic acid (4), ketopinoresinol (5) and daucosterol (6) from aerial parts of *A. sagittifolius* species collected from Vinh Hung commune, Vinh Loc district, Thanh Hoa province.

2. Experimental

2.1. General Experimental Procedures

The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F254). Compounds were visualized by spraying with Ce-Mo stain. The 1H-NMR (500 MHz) and 13C-NMR (125 MHz) spectra were recorded by a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard.

2.2. Plant Material

The plant materials of *Abelmoschus sagittifolius* were collected in the Bao mountain area, Vinh Hung commune, Vinh Loc district, Thanh Hoa province in 2020. Specimens were identified by Prof. Tran The Bach (Institute of Ecology and Biological Resources), and a voucher was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology with record number SB-2020.

2.3. Extraction and Isolation

The aerial parts of the *A. sagittifolius* were dried and ground into powder. The material (5.0 kg) was extracted in MeOH (20 L x 5 times, 24 hours/time) at room temperature. The combined extracts were evaporated *in vacuo* to obtain crude residue (0.5 L). The residue was suspended with water and was extracted successively with *n*-hexane and EtOAc. After removing the solvents, *n*-hexane residue (60 g) and EtOAc residue (10 g) were obtained, respectively.

The *n*-hexane residue (60 g) was separated on the silica gel column, eluted with the gradient *n*-hexane/EtOAc solvent system (0-100% EtOAc) to afford 5 fractions (HX1-HX5). The HX2 fraction (8.5 g) was subjected to normal phase silica gel CC and eluted with the *n*-hexane/EtOAc (10/1) to obtain compound 1 (17 mg). The HX4 fraction (13 g) was subjected to silica gel CC with the eluent system of *n*-hexane/EtOAc (4/1) to obtain compound 2 (7 mg).

The EtOAc residue (10 g) was separated in a normal phase silica gel CC, eluted with the *n*-hexane/EtOAc gradient solvent system (from 30% to 100% EtOAc) to obtain 7 fractions from E1-E7, respectively. The E1 fraction (0.6 g) was purified by normal phase silica gel CC with the eluent system of *n*-hexane/EtOAc (2/1) to obtain 3 sub-fractions E1.1-E1.3. The sub-fraction E1.2 (0.27 g) was purified by silica gel CC with *n*-hexane/EtOAc (2/1) to obtain compound 3 (30 mg). The E3 fraction (0.7 g) was purified by silica gel CC with *n*-hexane/EtOAc (1/1) elution system to afford 2 sub-fractions E3.1-E3.2. Purification of the E3.1 sub-fraction (0.37 g) obtained compound 4 (9 mg). The E4 fraction (1.2 g) was purified by silica gel CC with the eluent system CH2Cl2/MeOH (10/1) to obtain 3 sub-fractions E4.1-E4.3. The sub-fraction E4.1 (0.37g) was purified by Sephadex CC to obtain compound 5 (12 mg). The E6 fraction (2.1 g) was purified through a normal phase silica gel CC with CH2Cl2/MeOH (9/1) solvent system to obtain compound 6 (20 mg).

Sitostenone (1): White solid. ESI-MS: m/z 413 [M+H]+, molecular formula C26H43O (M = 412). 1H-NMR (500 MHz, CDCl3): δH (ppm) 5.71 (1H, s, H-4), 2.45-2.27 (5H, m), 2.01 (3H, m), 1.17 (3H, s, H-19), 0.91 (3H, d, J=6.5 Hz, H-21), 0.84 (3H, t, J=7.5 Hz, H-29), 0.83 (3H, d, J=7.0 Hz, H-27), 0.81 (3H, d, J=7.0 Hz, H-26), 0.70 (3H, s, H-18). 13C-NMR (125 MHz, CDCl3): δC (ppm) 199.6 (C-3), 171.6 (C-5), 123.7 (C-4), 56.0 (C-14), 55.9 (C-17), 53.8 (C-9), 45.8 (C-24), 42.4 (C-13), 39.6
Friedelin (2): White solid. ESI-MS: m/z 427 [M+H]^+; molecular formula C_{30}H_{50}O (M = 426). ^1H-NMR (500 MHz, CDCl_3): δ_H (ppm) 2.40-2.37 (1H, m, H-4), 2.30-2.23 (2H, m, H-2a, H-18), 1.99-1.94 (1H, m, H-2b), 1.18 (3H, s, Me-28), 1.05 (3H, s, Me-27), 1.01 (3H, s, Me-30), 1.00 (3H, s, Me-26), 0.95 (3H, s, Me-29), 0.88 (3H, d, J = 6.0 Hz, Me-23), 0.87 (3H, s, Me-25), 0.73 (3H, s, Me-24). ^13C-NMR (125 MHz, CDCl_3): δ_C (ppm) 213.0 (C-3), 59.5 (C-10), 58.2 (C-4), 53.1 (C-8), 42.8 (C-18), 42.1 (C-5), 41.5 (C-2), 41.3 (C-6), 39.7 (C-14), 39.2 (C-22), 38.3 (C-13), 37.4 (C-9), 36.0 (C-16), 35.6 (C-11), 35.3 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-15), 32.1 (C-28), 31.7 (C-30), 30.5 (C-17), 28.1 (C-20), 22.2 (C-1), 20.2 (C-26), 18.6 (C-27), 18.2 (C-7), 17.9 (C-25), 14.6 (C-24), 6.8 (C-23).

Vomifoliol (3): Colorless oil, [α]_D^{25} +190° (c 0.13, MeOH). ESI-MS: m/z 225 [M+H]^+; molecular formula C_{18}H_{30}O_3 (M = 224). ^1H-NMR (500 MHz, acetone-d_6): δ_H (ppm) 5.86 (1H, dd, J = 15.5, 4.5 Hz, H-8), 5.82 (1H, d, J = 15.5 Hz, H-7), 5.79 (1H, t, J = 1.5 Hz, H-5), 4.33 (1H, m, H-9), 2.42 (1H, d, J = 16.5 Hz, H-3), 2.08 (1H, d, J = 16.5 Hz, H-5), 1.88 (3H, s, H-13), 1.21 (3H, d, J = 6.5 Hz, H-10), 1.04 (3H, s, H-11), 1.00 (3H, s, H-12). ^13C-NMR (125 MHz, acetone-d_6): δ_C (ppm) 197.6 (C-4), 164.2 (C-6), 137.0 (C-8), 129.3 (C-7), 126.9 (C-5), 79.9 (C-1), 67.8 (C-9), 50.4 (C-3), 41.8 (C-2), 24.4 (C-11), 24.1 (C-10), 23.4 (C-12), 19.2 (C-13).

Vanillic acid (4): Pale yellow solid. ESI-MS: m/z 169 [M+H]^+; molecular formula C_9H_8O_4 (M = 168). ^1H-NMR (500 MHz, CD_2OD): δ_H (ppm) 7.58 (1H, d, J = 2.0 Hz, H-2), 7.56 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.85 (1H, d, J = 8.0 Hz, H-5), 3.91 (3H, s, OMe). ^13C NMR (125 MHz, CD_2OD) δ (ppm): 168.0 (COOH), 151.5 (C-3), 147.6 (C-4), 124.1 (C-1), 123.0 (C-6), 116.7 (C-2), 114.7 (C-5), 55.4 (OCH_3).

Ketopinoresinol (5): Light brown solid. ESI-MS: m/z 373 [M+H]^+; molecular formula C_{30}H_{40}O_7 (M = 372). ^1H-NMR (500 MHz, CD_2OD): δ_H (ppm) 6.98 (1H, d, J = 2.0 Hz, H-2), 6.96 (1H, d, J = 1.5 Hz, H-2'), 6.86 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.84 (1H, dd, J = 8.5, 1.5 Hz, H-6'), 6.81 (1H, d, J = 8.0 Hz, H-5), 6.81 (1H, d, J = 8.0 Hz, H-5'), 5.40 (1H, d, J = 4.0 Hz, H-7'), 5.25 (1H, d, J = 4.0 Hz, H-7), 4.31 (1H, dd, J = 9.5, 7.0 Hz, H-9a), 4.03 (1H, dd, J = 9.5, 4.5 Hz, H-9b), 3.89 (3H, s, OMe), 3.88 (3H, s, OMe), 3.67 (1H, dd, J = 9.0, 4.0 Hz, H-8'), 3.39 (1H, m, H-8). ^13C-NMR (125 MHz, CD_2OD): δ_C (ppm) 179.5 (C-9'), 149.4 (C-3), 149.2 (C-3'), 148.2 (C-4), 147.5 (C-4'), 133.2 (C-1), 132.4 (C-1'), 119.8 (C-6), 119.5 (C-6'), 116.5 (C-5), 116.1 (C-5'), 110.7 (C-2'), 110.6 (C-2), 87.2 (C-7), 85.1 (C-7'), 73.8 (C-9), 56.7 (OMe), 56.4 (OMe), 54.5 (C-8'), 51.1 (C-8).

Daucosterol (6): White powder. ESI-MS: m/z 577 [M+H]^+; molecular formula C_{30}H_{50}O_7 (M = 576). ^1H-NMR (500MHz, DMSO-d_6): δ (ppm) 5.34 (1H, br, H-6), 4.80 (3H, br, OH), 4.42 (1H, br, OH), 4.21 (1H, d, J = 8.0 Hz, H-1'), 3.63 (1H, d, J = 12.0 Hz, H-6'), 3.44 (1H, m, H-3), 3.40 (1H, m, H-6'), 3.12 (1H, d, J = 8.5 Hz, H-3'), 3.01-3.08 (2H, m, H-4', H-5'), 2.89 (1H, t, J = 8.0 Hz, H-2'), 2.36 (1H, dd, J = 10.0 Hz, H-4'), 2.12 (1H, m, H-4), 0.95 (3H, s, H-19), 0.90 (3H, d, J = 6.5 Hz, H-21), 0.82 (3H, t, J = 7.0 Hz, H-29), 0.81 (3H, d, J = 7.0 Hz, H-27), 0.79 (3H, d, J = 7.0 Hz, H-26), 0.64 (3H, s, H-18). ^13C-NMR (125 MHz, DMSO-d_6): δ (ppm) 140.6 (C-5), 121.1 (C-6), 100.8 (C-1'), 76.9 (C-3), 76.7 (C-5'), 76.7 (C-3'), 73.4 (C-2'), 70.1 (C-4'), 61.1 (C-6'), 56.1 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1(C-24), 41.8 (C-13), 39.2 (C-12), 38.3 (C-4), 36.8 (C-1), 36.2 (C-10), 35.4 (C-20), 33.3 (C-22), 31.4 (C-7), 31.3 (C-8), 29.2 (C-2), 28.7 (C-25), 27.7 (C-16), 25.4 (C-23), 23.8 (C-15), 22.6 (C-28), 20.5 (C-11), 19.6 (C-27), 19.0 (C-19), 18.9 (C-26), 18.6 (C-21), 11.7 (C-18), 11.6 (C-29).
3. Results and Discussion

Compound 1 was obtained as a white solid. The $^1$H-NMR spectrum shows the characteristic of sterol compounds with signals of 6 methyl groups at $\delta_H$ 1.17 (3H, s, H-19), 0.91 (3H, d, J = 6.5 Hz, H-21), 0.84 (3H, t, J = 7.5 Hz, H-29), 0.83 (3H, d, J = 7.0 Hz, H-27), 0.81 (3H, d, J = 7.0 Hz, H-26), 0.70 (3H, s, H-18). There is a signal of an olefinic proton at $\delta_H$ 5.71 (1H, s, H-4). The $^{13}$C-NMR, DEPT spectrum shows a signal of 29 carbons, including 6 CH$_3$ group signals, 11 CH$_2$ group signals, and 8 CH group signals (in which the CH=C signal is at position 123.7 (C-4)), four quaternary C signals including a carbonyl group at $\delta_C$ 199.6 (C-3). The NMR spectral data suggest that compound 1 is a sterol compound. Comparison of the spectral data with the published sitostenone data showed agreement [8], so compound 1 was identified as sitostenone.

The compound 2 was obtained as a white solid. In the $^1$H-NMR spectrum, there are typical signals of friedelane triterpene with signals of 8 methyl groups, including 7 singlet methyl groups at $\delta_H$ 1.18 (3H, s, Me-28), 1.05 (3H, s, Me-27), 1.01 (3H, s, Me-30), 1.00 (3H, s, Me-26), 0.95 (3H, s, Me-29), 0.87 (3H, s, Me-25), 0.73 (3H, s, Me-24) and 1 methyl doublet group at $\delta_H$ 0.88 (3H, d, J = 6.0 Hz, Me-23). The $^{13}$C-NMR and DEPT spectra of compound 2 give signals of 30 carbons including 8 methyl groups at $\delta_C$ 32.1 (C-28), 31.7 (C-30), 20.2 (C-26), 18.6 (C-27), 18.2 (C-7), 17.9 (C-25), 14.6 (C-24) and 6.8 (C-23), 11 methylene groups, 5 methine groups and 6 quaternary carbon signals including the C=O group at $\delta_C$ 213.0 (C-3). From the ESI-MS mass spectrum of compound 2 showed ion peak at m/z 427 [M+H]$^+$, combined with $^1$H and $^{13}$C NMR spectral data and comparison with all data of spectra in the previously study [9], it can be concluded that compound 2 was friedelin with molecular formula of C$_{30}$H$_{50}$O$^\text{a}$.

The compound 3 was isolated from the EtOAc extract as colorless oil. In the $^1$H-NMR spectrum showed the signal of 3 olefinic
protons at $\delta_H 5.86$ (1H, dd, $J = 15.5, 4.5$ Hz, H-8), 5.82 (1H, d, $J = 15.5$ Hz, H-7), 5.79 (1H, t, $J = 1.5$ Hz, H-5), an oxymethylene group at $\delta_H 4.33$ (1H, m, H-9) and 4 methyl groups including 3 singlets at $\delta_H 1.88$ (3H, s, H-13), 1.04 (3H, s, H-11), 1.00 (3H, s, H-12) and a doublet at $\delta_H 1.21$ (3H, d, $J = 6.5$ Hz, H-10). In the $^{13}$C-NMR and HSQC spectra, there were 13 carbon signals including 1 carbonyl group at $\delta_C 197.6$ (C-4), 4 olefinic carbon signals at $\delta_C 164.2$ (C-6), 137.0 (C-8), 129.3 (C-7), 126.9 (C-5), carbon signal bound to oxygen and oxymethylene group at $\delta_C 79.9$ (C-1), 67.8 (C-9), respectively and 4 methyl group signals at $\delta_C 24.4$ (C-11), 24.1 (C-10), 23.4 (C-12), 19.2 (C-13). From the ESI-MS spectrum, there was pseudo-molecular ion peak at $m/z$ 226, combined with $^1$H, $^{13}$C spectral data; suggested that compound 3 was a megastigmane structure with molecular formula of $C_{20}H_{26}O_4$. By analyzing the spectral data of compound 3, and comparing the optical rotation value,$^{10}$ 3 was determined as vomifoliol.$^{11}$

Compound 4 was isolated as a pale yellow solid. The $^1$H-NMR spectrum exhibited the signals of three aromatic protons of the ABX system at $\delta_H 7.58$ (1H, d, $J = 2.0$ Hz, H-2), 7.56 (1H, dd, $J = 2.0$, 8.0 Hz, H-6), 6.85 (1H, d, $J = 8.0$ Hz, H-5) and 1 methoxy group. The $^{13}$C-NMR spectrum showed carboxylic acid group signal at $\delta_C 168.0$ (COOH) and 6 aromatic carbon signals at $\delta_C 151.5$ (C-3), 147.6 (C-4), 124.1 (C-1), 123.0 (C-6), 116.7 (C-2), 114.7 (C-5), methoxy group at $\delta_C 55.4$ (OCH$_3$). In the ESI-MS spectrum revealed pseudo-molecular ion peak $m/z$ 169 [M+H]$^+$, combined with $^1$H and $^{13}$C NMR spectra and comparison with NMR spectral data, ESI-MS spectrum to the reported literature.$^{12}$ It can be concluded that compound 4 was vanillic acid with molecular formula of $C_{13}H_{12}O_2$.

Compound 5 was obtained as a light brown solid. In the $^1$H-NMR spectrum, signals of aromatic protons of two ABX systems appeared at $\delta_H 6.98$ (1H, d, $J = 2.0$ Hz, H-2), 6.86 (1H, dd, $J = 8.0$, 2.0 Hz, H-6), 6.81 (1H, d, $J = 8.0$ Hz, H-5) and 6.96 (1H, d, $J = 1.5$ Hz, H-2), 6.84 (1H, dd, $J = 8.5$, 1.5 Hz, H-6') and 6.81 (1H, d, $J = 8.0$ Hz, H-5'). 2 protons at $\delta_H 5.40$ (1H, d, $J = 4.0$ Hz, H-7'), 5.25 (1H, d, $J = 4.0$ Hz, H-7), and 1 oxymethylene group at $\delta_H 4.31$ (1H, dd, $J = 9.5$, 7.0 Hz, H-9a), 4.03 (1H, dd, $J = 9.5$, 4.5 Hz, H-9b), 2 methoxy groups at $\delta_H 3.89$ (3H, s, OMe), 3.88 (3H, s, OMe) and 2 methine groups at $\delta_H 3.67$ (1H, dd, $J = 9.0$, 4.0 Hz, H-8') and 3.39 (1H, m, H-8). The $^{13}$C-NMR and HSQC spectra showed signals of 20 carbons including 2 methoxy groups at $\delta_C 56.7$ (OMe) and 56.4 (OMe). The remaining 18 carbon signals suggest a lignan structure with 12 aromatic ring signals from $\delta_C 110.6$-$149.6$, 1 carbonyl group signal at $\delta_C 179.5$ (C-9'), oxymethylene group at $\delta_C 73.8$ (C-9), 2 oxymethylene groups at $\delta_C 87.2$ (C-7) and 85.1 (C-7'), 2 methine groups at $\delta_C 54.5$ (C-8') and 51.1 (C-8). From analysis of $^1$H, $^{13}$C spectra, combined with the protonated molecular ion peak at $m/z$ 373 [M+H]$^+$ observed in the ESI-MS spectrum and comparison with NMR spectral data and ESI-MS spectrum to the reported literature,$^{13}$ it can be concluded that compound 5 was ketopinoresinol with molecular formula of $C_{20}H_{26}O_7$.

Compound 6 was obtained as a white powder. The $^1$H-NMR spectrum appeared signals specific to sterol glycoside compounds, in which the sterol compound has an olefinic proton signal at $\delta_H 5.34$ (1H, br), a hydroxymethine at $\delta_H 3.44$ (1H, m) and 6 methyl groups at $\delta_H 0.95$ (3H, s), 0.91 (3H, d, $J = 6.5$ Hz), 0.82 (3H, t, $J = 7.0$ Hz), 0.81 (3H, d, $J = 7.0$ Hz), 0.79 (3H, d, $J = 7.0$ Hz) and 0.64 (3H, s). The sugar moiety has anomeric proton at $\delta_H 4.21$ (1H, d, $J = 8.0$ Hz), the CH$_2$OH and CHO group signals appeared at $\delta_H 3.63$ (1H, d, $J = 12$ Hz), 3.40 (1H, m, overlapped), 3.12 (1H, d, $J = 8.5$ Hz), 3.01-3.08 (2H, m), 2.89 (1H, t, $J = 8.0$ Hz). The $^{13}$C-NMR and DEPT spectra show that compound 6 has 35 carbon signals including 6 CH$_3$ groups, 12 CH$_2$ groups, 14 CH groups and 3 quaternary carbons, in which the signal of the double bond appears at $\delta_C 140.6$ (C-5), 121.1 (C-6). The anomeric carbon signal appeared at $\delta_C 100.8$ ppm. Signals of the glucose groups at $\delta_C 76.7, 76.7, 73.4, 70.1$ and 61.1. Signals of 6 methyl groups
appeared at \( \delta_c \) 19.6, 19.0, 18.9, 18.6, 11.7 and 11.6 ppm. From analysis of \( ^1H, ^13C \) NMR spectra, combined with the pseudo-molecular ion peak at \( m/z \) 577 [M+H]+ observed in the ESI-MS spectrum and comparison with NMR spectral data and ESI-MS spectrum to the previous study [14], it can be concluded that compound 6 was daucosterol with the molecular formula of \( C_{35}H_{60}O_6 \).

4. Conclusion

From the aerial parts of \( A. \) sagittifolius (Kurz) Merr., six compounds were isolated and structurally determined as sitostenone (1), friedelin (2), vomifoliol (3), vanilic acid (4), ketopinoresinol (5) and daucosterol (6). Compounds 1-2 and 4-5 were isolated for the first time from \( A. \) sagittifolius while compound 3 was found in roots of this species.

Acknowledgements

This research is funded by Thanh Hoa People’s Committee under project in 2020.

References