Hue University Journal of Science: Natural Science; ISSN 1859-1388

Vol. 126, No. 1C, 2017, P. 101-106; DOI: 10.26459/hueuni-jns.v126i1C.4602

LONG-CHAIN COMPOUNDS ISOLATED FROM LAC TIEN (*PASSIFLORA FOETIDA* L.)

Nguyen Chi Bao[1]*, Pham Viet Ty[2]

[1] Hue University, 3 Le Loi St., Hue, Vietnam;
[2] University of Education, Hue University, 34 Le Loi St., Hue, Vietnam

Abstract. Three fatty acids, namely stearic acid (**1**), linoleic acid (**2**), linolenic acid (**3**) together with triacontan-1-ol (**4**), α-tocopherol (**5**) were isolated from the aerial parts of *Passiflora foetida* L. Their structure was elucidated using the spectroscopic methods, viz FT–IR, ESI–MS, NMR and by comparing with the published data. Compounds **4** and **5** were found for the first time from *Passiflora* genus.

Keywords: *Passiflora foetida*, stearic acid, linoleic acid, linolenic acid, triacontan-1-ol, α-tocopherol

1 Introduction

The genus *Passiflora* comprises about 500 species and is the largest in family Passifloraceae. The species of this genus are distributed in the warm temperate and tropical regions of the New World; they are much rarer in Asia, Australia, and tropical Africa [1,2]. *P. foetida* is South American in origin, which has been spread to many tropical areas [2]. In Vietnam, *P. foetida* is a plant growing wildly everywhere, especially in some provinces such as Hoa Binh, Thai Nguyen, Bac Giang, Quang Binh, Thua Thien Hue, Da Nang and Quang Nam [3]. The ethnobotanical views of *P. foetida* reports that the decoction of leaves and fruits is used for the treatment of asthma and biliousness; leaf and root decoction is used for emmenagogue and hysteria; leaf paste is applied on the head for giddiness and headache [2,4,5]. Bioactivity studies of *P. foetida* have indicated that it possesses analgesic, antidiarrhoeal, anti-inflammatory and cytotoxic activities. The phytoconstituents of this plant contain reducing sugars, alkaloids, flavonoids, tannins, steroids, gums, glycoside, cyanogenic compounds, and polyketides [4,5].

In the previous study, we reported the isolation and structural elucidation of some constituents from the methanol extract of *P. foetida* [6]. In this paper, we report the isolation and identification of three fatty acids, one long-chain alcohol and α-tocopherol from the *n*-hexane and dichloromethane extracts of *P. foetida*.

2 Experiments

2.1 General experimental procedures

The electrospray ionization (ESI) mass spectra were recorded on an Agilent LC–MSD–Trap SL spectrometer. The FT–IR spectra were recorded on a Shimadzu IR 8400 Prestige spectrometer

* *Corresponding: baosphoa@gmail.com*

Submitted: 21-11-2017; Revised: 7-2-2018; Accepted: 17-4-2018

with KBr discs. The ^{1}H–NMR (500 MHz) and ^{13}C–NMR (125 MHz) spectra were measured on a Bruker Avance 500 MHz; TMS was used as an internal reference.

Thin layer chromatography (TLC) was carried out on aluminum plates precoated with Si 60 F_{254} (Merck, Germany). The compounds were detected from their UV absorbance and with vanillin/H_2SO_4 reagent. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC RP–18 resins (30–50 μm, Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan).

2.2 Plant material, extraction and isolation

The aerial parts of *P. foetida* were collected in Phu Loc, Thua Thien Hue in January, 2016. The dried and powdered plant material (1.6 kg) was exhaustively extracted with *n*-hexane, dichloromethane, ethyl acetate and methanol at room temperature. After filtration, the solvents were removed using evaporation, and four crude extracts were obtained.

The *n*-hexane extract (10 g) was chromatographed on the silica gel column, eluted with *n*-hexane:acetone (4:1, v/v) to give seven fractions (H1–H7). Fraction H2 (225 mg) was separated into four subfractions (H2.1–H2.4) on the silica gel column, eluted with *n*-hexane:acetone (50:1, v/v) to yield **1** (10 mg) and **2** (12 mg). Sub-fraction H2.1 (43 mg) was then purified on the silica gel column eluted with *n*-hexane:acetone (50:1, v/v), followed by YMC RP–18 CC with acetone:methanol (2:1, v/v) to afford **5** (8 mg). Fraction H4 (150 mg) was chromatographed on the silica gel column, using *n*-hexane:acetone (20:1, v/v) as an eluent, to yield **3** (15 mg).

Similarly, the dichloromethane extract (14.9 g) was chromatographed on the silica gel column, eluted with the *n*-hexane:acetone gradient system (95:5-0:100, v/v) to obtain forty-five fractions, D1–D45. Compound **4** (43 mg) was isolated from fraction D8 after recrystallized repeatedly in dichloromethane.

Compound **1**: White amorphous powder; ESI–MS: *m*/*z* 285 $[M+H]^+$; ^{1}H–NMR ($CDCl_3$, 500 MHz): 2.34 (2H, t, 7.5 Hz, H-2); 1.63 (2H, m, H-3); 1.26 (28H, m, H-4~H-17); 0.88 (3H, t, 6.5 Hz, H-18); ^{13}C–NMR ($CDCl_3$, 125 MHz) (Table 1).

Compound **2**: Colorless oil; ^{1}H–NMR ($CDCl_3$, 500 MHz): 5.32–5.39 (4H, m, H-9, H-10, H-12, H-13); 2.77 (2H, t, 7.0 Hz, H-11); 2.34 (2H, t, 7.5 Hz, H-2); 2.04 (4H, m, H-8, H-14); 1.63 (2H, m, H-3); 1.26-1.34 (14H, m, H-4~H-7, H-15~H-17); 0.88 (3H, t, 7.0 Hz, H-18); ^{13}C–NMR ($CDCl_3$, 125 MHz) (Table 1).

Compound **3**: Colorless oil; ESI–MS: *m*/*z* 277 $[M-H]^-$; ^{1}H–NMR ($CDCl_3$, 500 MHz): 5.30–5.41 (6H, m, H-9, H-10, H-12, H-13, H-15, H-16); 2.81 (4H, t, 6.5 Hz, H-11, H-14); 2.34 (2H, t, 7.5 Hz, H-2); 2.06 (4H, m, H-8, H-17); 1.63 (2H, m, H-3); 1.26–1.35 (8H, m, H-4~H-7); 0.98 (3H, t, 7.5 Hz, H-18); ^{13}C–NMR ($CDCl_3$, 125 MHz) (Table 1).

Compound **4**: White amorphous powder; FT–IR (KBr) ν_{max} (cm^{-1}): 3421.7; 2916.4; 2846.9; 1060.9; 721.4 ; ESI–MS: *m*/*z* 439 $[M+H]^+$; ^{1}H–NMR ($CDCl_3$, 500 MHz): 3.64 (2H, t, 6.5 Hz, H-1); 1.57 (2H, t, 6.8 Hz, H-2); 1.26 (m, H-3~H-29); 0.88 (3H, t, 6.0 Hz, H-30); ^{13}C–NMR ($CDCl_3$, 125 MHz): 63.12 (C-1); 32.85 (C-2); 22.71–31.95 (C-3~C-29); 14.12 (C-30).

Compound **5**: Yellow oil; ESI–MS: *m*/*z* 431 $[M+H]^+$; ^1H–NMR ($CDCl_3$, 500 MHz): 4.17 (1H, s, OH); 2.60 (2H, t, 7.0 Hz, H-4); 2.16 (3H, s, H-7a); 2.11 (6H, s, H-5a, H-8b); 1.77 (2H, m, H-3); 1.23 (3H, s, H-2a); 0.87 (6H, d, 7.0 Hz, H-12′a, H-13′); 0.83–0.85 (6H, d, 6.5 Hz, H-4′a, H-8′a).

Table 1. 13C–NMR (125MHz) data of compounds **1**, **2, 3** and reference compounds

C	**1** ($CDCl_3$)	**Stearic acid ($CDCl_3$) [7]**	**2** ($CDCl_3$)	**Linoleic acid ($CDCl_3$) [10]**	**3** ($CDCl_3$)	**Linolenic acid ($CDCl_3$) [11]**
1	179.44	179.9	180.09	180.55	180.02	180.43
2	33.96	34.2	34.06	34.15	34.04	34.13
3	24.70	24.8	24.69	24.70	24.66	24.69
4	29.07–29.70	29.3–29.9	29.07	29.08	29.03	29.09
5	29.07–29.70	29.3–29.9	29.14	29.12	29.07	29.13
6	29.07–29.70	29.3–29.9	29.35	29.40	29.56	29.62
7	29.07–29.70	29.3–29.9	29.69	29.63	29.14	29.21
8	29.07–29.70	29.3–29.9	27.18	27.22	27.20	27.23
9	29.07–29.70	29.3–29.9	130.02	130.02	131.96	131.85
10	29.07–29.70	29.3–29.9	128.08	128.12	127.77	127.16
11	29.07–29.70	29.3–29.9	25.63	25.67	25.63	25.56
12	29.07–29.70	29.3–29.9	127.91	129.95	128.30	128.26
13	29.07–29.70	29.3–29.9	130.21	130.21	128.26	128.22
14	29.07–29.70	29.3–29.9	27.21	27.25	25.54	25.65
15	29.07–29.70	29.3–29.9	29.24	29.19	127.13	127.80
16	31.93	32.2	31.92	31.58	130.24	130.14
17	22.69	22.9	22.68	22.62	20.55	20.56
18	14.10	14.1	14.09	14.09	14.25	14.26

3 Results and discussion

In the positive ESI–MS, compound **1** showed a pseudo molecular ion peak at *m*/*z* 285 $[M+H]^+$, and together with NMR data, the molecular formula of **1** was established as $C_{18}H_{36}O_2$. The ^1H–, ^{13}C–NMR and DEPT data showed that compound **1** was a saturated unbranched-chain fatty acid. It contained a carboxyl group at δ_C 179.44 (C-1), one methyl group at δ_H 0.88 (3H, m, 6.5 Hz, H-18) and δ_C 14.10 (C-18), one methylene group at the α position of the carboxyl group [δ_H 2.34 (2H; t; 7.5 Hz, H-2) and δ_C 33.96 (C-2)]; one methylene group at the β position of the carboxyl group [δ_H 1.63 (2H; m, H-3) and δ_C 24.70 (C-3)]; other methylene groups [δ_H 1.26 (28H; m; H-4~H-17) and δ_C 29.07–29.70 (C4~C15), 31.93 (C-16) and 22.69 (C-17)]. According to the evidence above and the reported data [7], compound **1** was identified as stearic acid (Figure 1).

Compound **2** was predicted as an unsaturated straight chain fatty acid from the signals in the ^1H–, ^{13}C–NMR and DEPT spectra. The ^1H–NMR spectrum of compound **2** indicated the appearance of four olefinic protons from 5.32 to 5.39 ppm (4H, m, H-9, H-10, H-12, H-13), two protons attached to the *bis*-allylic carbons at 2.77 (2H, t, 7.0 Hz, H-11), two protons in the methylene group at the α position of the carboxyl group at 2.34 (2H, t, 7.5 Hz, H-2); four protons attached to the allylic carbons at 2.04 (4H, m, H-8, H-14) and the terminal methyl group protons at 0.88 (3H, t, 7.0 Hz, H-18) [8, 9]. The *Z* configuration of two double bonds was deduced from the small coupling constants. The ^{13}C–NMR and DEPT spectra showed the presence of a carboxyl group at δ_C 180.09 (C-1), four methine olefinic carbons at 130.02 (C-9), 128.08 (C-10), 127.91 (C-12), 130.21 (C-13); one methyl group at 14.09 (C-18). The remained signals from 22.57 to 34.06 ppm belonged to the methylene groups. From this evidence and along with that in the literature [8,10], compound **2** was deduced as linoleic acid.

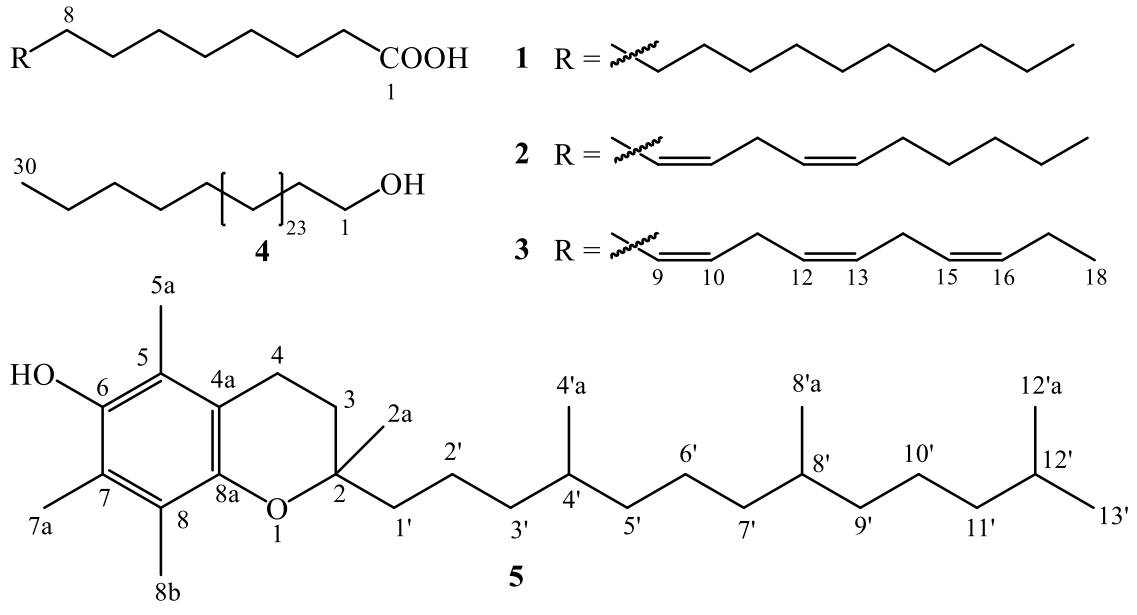

Fig. 1. Structure of **1–5** from the aerial parts of *P. foetida*

The molecular formula of **3** was deduced as $C_{18}H_{30}O_2$ on the basis of the ESI–MS (at *m*/*z* 277 $[M-H]^-$) and NMR data. The signals in the ^{1}H–NMR spectrum of compound **3** were similar to those of compound **2**, suggesting that compound **3** was also an unsaturated straight-chain fatty acid. The presence of a carboxyl group at δ_C 180.02 (C-1); and three double bonds was observed from six olefinic protons from 5.30 to 5.41 ppm (6H, m, H-9, H-10, H-12, H-13, H-15, H-16), six methine olefinic carbons at 130.24 (C-9), 127.77 (C-10), 128.30 (C-12), 128.26 (C-13), 127.13 (C-15), 131.96 (C-16); four protons attached to the *bis*-allylic carbons at 2.81 (4H, t, 6.5 Hz, H-11, H-14), two protons in the methylene group at the α position of the carboxyl group at 2.34 (2H, t, 7.5 Hz, H-2); four protons attached to the allylic carbons at 2.06 (4H, m, H-8, H-17) and the terminal methyl group at 0.98 (3H, t, 7.5 Hz, H-18), 14.25 (C-18). From the analysis of the spectroscopic data and the data in the literature [11], the structure of compound **3** was determined as linolenic acid.

The molecular formula of **4** was established as $C_{30}H_{62}O$ from the $[M+H]^+$ peak at *m*/*z* 439 in the positive ESI–MS. The FT–IR spectrum of compound **4** showed the absorptions of some functional groups: O–H (3421.7 cm^{-1}), C_{sp3}–H (2846.9, 2916.4 cm^{-1}), C–O (1060.9 cm^{-1}) and a long-chain band of CH_2 groups (721.4 cm^{-1}). The ^{1}H– and ^{13}C–NMR spectra of compound **4** showed the presence of one oxygenated methylene group at δ_H 3.64 (2H, t, 6.5 Hz, H-1) and δ_C 63.12 ppm. The signal at δ_H 1.57 (2H, t, 6.8 Hz, H-2) belonged to two methylene protons at C-2, corresponding to the signal of C-2, linked directly to the oxymethylene group, at δ_C 32.85 (C-2). Besides, the resonance signal in the upfield at δ_H 0.88 (3H, t, 6.0 Hz, H-30) belonged to the methyl protons at C-30 and at 1.26 (m, H-3~H29) of the overlapped methylene groups. Thus, compound **4** was identified to be triacontan-1-ol.

In the positive ESI–MS, compound **5** showed a pseudo molecular ion peak at *m*/*z* 431 $[M+H]^+$ corresponding to the molecular formula of $C_{29}H_{50}O_2$. The ^{1}H–NMR spectrum indicated the signals of three methyl singlets connected to the aromatic ring at 2.16 (3H, s, H-7a); 2.11 (6H, s, H-5a, H-8b); four methyl doublets at 0.87 (6H, d, 7 Hz, H-12'a, H-13'); 0.83-0.85 (6H, d, 6.5 Hz, H-4'a, H-8'a); one methyl singlet at 1.23 (3H, s, H-2a). In addition, its ^{1}H–NMR spectrum also showed the signals of one methylene group attached to the aromatic ring at 2.60 (2H, t, 7.0 Hz, H-4); one hydroxyl group at the C-6 position in the aromatic ring at 4.17 (1H, s, OH). The data above and other spectral data [12] led to conclude that compound **5** was α-tocopherol.

4 Conclusion

Five known compounds, namely stearic acid, linoleic acid, linolenic acid, triacontan-1-ol, and α-tocopherol were isolated from the aerial parts of *P. foetida*. To the best of our knowledge, triacontan-1-ol and α-tocopherol were isolated for the first time from the genus *Passiflora* and stearic acid was isolated for the first time from *P. foetida*. The structural identification of the isolated compounds was conducted by using the combination of spectroscopic data including IR, MS, 1D–NMR, and the information from the literature.

References

1. Dhawan K., Dhawan S., Sharma A. (2004), Passiflora: a review update, *Journal of Ethnopharmacology*, 94, 1-23.
2. Sasikala V., Saravanan S., Parimelazhagan T. (2011), Analgesic and anti-inflammatory activities of *Passiflora foetida* L., *Asian Pacific Journal of Tropical Medicine*, 4(8), 600–603.
3. Bich D. H. et al. (2004), *Medicinal animals and plants in Vietnam*, Hanoi Science and Technology Publishing House, 138–140.
4. Mohanasundari C., Natarajan D., Srinivasan K., Umamaheswari S. and Ramachandran A. (2007), Antibacterial properties of *Passiflora foetida* L. - A common exotic medicinal plant, *African Journal of Biotechnology*, 6(23), 2650–2653.
5. Asadujjaman Md., Mishuk A. U., Hossain Md. A., Karmakar U. K. (2014), Medicinal potential of *Passiflora foetida* L. plant extracts: Biological and pharmacological activities, *Journal of Integrative Medicine*, 12(2), 121–126.
6. Nguyen Chi Bao, Pham Viet Ty (2017), Compounds isolated from methanol extract of Lac tien *(Passiflora foetida* L.), *Hue University Journal of Science*, 126(1A), 133–139.
7. Sun B. N., Shen H. D., Wu H. X., Yao L. X., Cheng Z. Q. and Diao Y. (2014), Determination of Chemical Constituents of the Marine Pulmonate Slug, Paraoncidium reevesii, *Tropical Journal of Pharmaceutical Research*, 13(12), 2071–2074.
8. Nguyen Thi Hoai (2017), *Studies of anticancer agents from Hedyotis genus (Rubiaceae) in Vietnam*, Ministry of Education and Training Project, Code B2015–DHH–126.
9. Knothe G., Kenar J. A. (2004), Determination of the fatty acid profile by ^{1}H-NMR spectroscopy, *European Journal of Lipid Science and Technology*, 106, 88–96.
10. Butovich I. A., Lukyanova S. M. (2008), Inhibition of lipoxygenases and cyclooxygenases by linoleyl hydroxamic acid: comparative in vitro studies, *Journal of Lipid Research*, 49, 1284–1294.
11. Yang Q., Cao W., Zhou X., Cao W., Xie Y. and Wang S. (2014), Anti-thrombotic effects of α-linolenic acid isolated from *Zanthoxylum bungeanum* Maxim seeds, *BMC Complementary and Alternative Medicine*, 14(348), 1–8.
12. Odinokov V. N., Spivak A. Y., Emelyanova G. A., Mallyabaeva M. I., Nazarova O. V., and Dzhemilev U. M. (2003), Synthesis of α-tocopherol (vitamin E), vitamin K1-chromanol, and their analogs in the presence of aluminosilicate catalysts Tseokar-10 and Pentasil, *Arkivoc*, 13, 101–118.