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Chemical constituents from methanolic extract of *Solanum procumbens* Lour (Solanaceae)

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Abstract:

***Solanum procumbens* L. (Solanaceae) were extracted respectively with *n*-hexane, ethyl acetate, and methanol to obtain the corresponding extracts. The methanol-soluble extract was acidified by adding HCl 0.5M; this was followed by liquid-liquid partition with $CHCl_3$ to afford $CHCl_3$-soluble fraction. From this fraction, four compounds were isolated and identified as ergosterol peroxide (1), 9,11-dehydroergosterol peroxide (2), *N*-(*N*-benzoyl-L-phenylalanyl)-L-phenylalanol (3), and moupinamide (4). Their structures were elucidated based on the NMR spectroscopic analysis and comparison with the literature. All isolated compounds were first reported from *S. procumbens*.**

Keywords: **ergosterol peroxide, moupinamide, Solanaceae, *Solanum procumbens.***

Classification number: **2.2**

Introduction

Solanum procumbens L., which belongs to the genus *Solanum* (Solanaceae), was collected at Tinh Bien district, An Giang province, Vietnam. In traditional medicine, the entire plant of *S. procumbens* is used for curing arthritis and hepatitis treatments as well as for supportive treatment of the liver [1]. Based on the literature, *S. procumbens* demonstrated potential antimicrobial activities [2]. The previous investigations regarding chemical constituents of *S. procumbens* precipitated the isolation of flavonoids, steroids, phenols, coumarins, and glycoalkaloids [3]. This paper reports the extraction and structural elucidation of isolated compounds from methanol extract of *S. procumbens*. As a result, four compounds were identified as ergosterol peroxide (**1**), 9,11-dehydroergosterol peroxide (**2**), *N*-(*N*-benzoyl-L-phenylalanyl)-L-phenylalanol (**3**), and moupinamide (**4**).

Experimental

Plant materials

The entire plant of *S. procumbens* was collected at Tinh Bien district, An Giang province, Vietnam. The plant was identified by the Institute of Drug Quality Control, Ho Chi Minh city, Vietnam. The plant sample was identified by Dr. rer. nat. Anh Tuan Dang-Le, Lab. of Botany, Department of Ecology and Evolutionary Biology, Faculty of Biology-Biotechnology, University of Science, Vietnam National University, Ho Chi Minh city.

General procedures

NMR spectra were taken using a Bruker Avance III 500 spectrometer, with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in ppm. All of these spectroscopic data were recorded in VNUHCM-University of Science. Column chromatography was performed on silica gel (Kieselgel 60, 40-63 μm, Merck)

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and Lichroprep RP_{18} (40-63 µm, Merck). Analytical and preparative TLC (Thin-Layer Chromatography) were performed on precoated Kieselgel $60F_{254}$ or RP_{18} plates (0.25 mm, Merck).

Extraction and isolation of compounds

The entire plants of *S. procumbens* were dried and cut into small pieces (4.0 kg) and then were Soxhlet extracted respectively with *n*-hexane, ethyl acetate, and methanol to yield the corresponding extracts. The methanol-soluble extract (89.0 g) was acidified by acid HCl 0.5M (pH 2-3). Subsequently, the water was added, and the liquid was then partitioned with $CHCl_3$. The $CHCl_3$-soluble extract was evaporated under reduced pressure to afford $CHCl_3$ fraction (7.8 g). This fraction was subjected to a normal phase silica gel column chromatography eluted with *n*-hexane/EtOAc (0-100% EtOAc) and EtOAc/MeOH (0-30% MeOH) mixtures to afford nine fractions (**C1-C9**). Fraction **C2** (378 mg) was repeatedly chromatographed over a silica gel column and eluted with *n*-hexane/EtOAc/Et_3N (80:10:10) mixtures to produce five fractions (**C2.1-C2.5**). Fraction **C2.4** was purified by preparative TLC with *n*-hexane/EtOAc (80:20) as an eluent, to yield compounds **1** (7.0 mg) and **2** (3.5 mg). Fraction **C3** (675 mg) was chromatographed over a silica gel column eluted with petroleum ether/EtOAc (0-50% EtOAc) mixtures to obtain compound **3** (12.0 mg). Fraction **C4** (798 mg) was separated by column chromatography eluted with $CHCl_3$/MeOH (95:5) mixtures to create compound **4** (8.6 mg) (Fig. 1).

Ergosterol peroxide (**1**): ^{1}H NMR (acetone-d_6, 500 MHz): δ_H (ppm) 6.49 (1H, *d*, *J* = 8.5 Hz, H-7), 6.23 (1H, d, *J* = 8.5 Hz, H-6), 5.28 (1H, *dd*, *J* = 15.3, 7.5 Hz, H-23), 5.21 (1H, *dd*, *J* = 15.3, 8.2 Hz, H-22), 3.76 (1H, *m,* H-3), 3.70 (1H, *d*, *J* = 5.0 Hz, 3-OH), 1.97 (1H, *dd*, *J* = 9.1, 2.9 Hz, H-16a), 1.93 (2H, *m*, H-4a, -11a), 1.90 (1H, *m*, H-4b), 1.87 (1H, *m*, H-24), 1.74 (1H, *m*, H-12a), 1.67 (2H, *dt*, *J* = 13.2, 3.4 Hz, H-1), 1.54 (2H, *m*, H-15), 1.50 (2H, *m*, H-12b, -14), 1.48 (1H, *m*, H-25), 1.41 (1H, *m*, H-9), 1.27 (1H, *m*, H-17), 1.25 (2H, *m*, H-11b, -16b), 1.03 (3H, *d*, *J* = 6.4 Hz, H-21), 0.93 (3H, *d*, *J* = 6.8 Hz, H-28), 0.89 (3H, *s*, H-19), 0.86 (3H, *s*, H-18), 0.85 (3H, *d*, *J* = 6.7 Hz, H-27), 0.84 (3H, *d*, *J* = 6.7 Hz, H-26). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C (ppm) 136.6 (C-6), 136.5 (C-22), 133.0 (C-23) 131.3 (C-7), 82.5 (C-5), 79.5 (C-8), 66.5 (C-3), 57.1 (C-17), 52.8 (C-14), 52.6 (C-9), 45.3 (C-13), 43.8 (C-24), 40.6 (C-20), 40.4 (C-12), 38.1 (C-4), 37.9 (C-10), 35.7 (C-1), 33.9 (C-25), 31.1 (C-2), 29.1 (C-16), 24.1 (C-15), 21.4 (C-11, -21), 20.3 (C-26), 20.0 (C-27), 18.5 (C-19), 18.1 (C-28), 13.3 (C-18). HRESIMS *m/z* 451.3183 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_3Na$, 451.3188).

9,11-Dehydroergosterol peroxide (**2**): ^{1}H NMR (acetone-d_6, 500 MHz): δ_H (ppm) 6.62 (1H, *d*, *J* = 8.5 Hz, H-7), 6.29 (1H, d, *J* = 8.5 Hz, H-6), 5.46 (1H, *dd*, *J* = 6.0, 2.0 Hz, H-11), 5.29 (1H, *dd*, *J* = 15.3, 7.4 Hz, H-22), 5.22 (1H, *dd*, *J* = 15.3, 8.2 Hz, H-23), 3.81 (1H, *m,* H-3), 1.09 (3H, *s*, H-19), 1.03 (3H, *d*, *J* = 6.6 Hz, H-21), 0.94 (3H, *d*, *J* = 6.8 Hz, H-28), 0.86 (3H, *d*, *J* = 6.6 Hz, H-27), 0.84 (3H, *d*, *J* = 6.6 Hz, H-26), 0.78 (3H, *s*, H-18). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C (ppm) 136.8 (C-7), 136.4 (C-22), 133.0 (C-23), 131.3 (C-6), 119.5 (C-11), 116.0 (C-9), 83.1 (C-8), 78.5 (C-5), 66.1 (C-3), 56.7 (C-17), 49.3 (C-14), 44.3 (C-13), 43.7 (C-24), 42.0 (C-12), 40.8 (C-20), 38.1 (C-10), 37.2 (C-4), 33.9 (C-25), 33.5 (C-1), 31.7 (C-2), 29.5 (C-16), 25.9 (C-19), 21.5 (C-15), 21.1 (C-21), 20.3 (C-26), 19.9 (C-27), 18.0 (C-28), 13.3 (C-18). HRESIMS *m/z* 449.3027 $[M+Na]^+$ (calcd for $C_{28}H_{42}O_3Na$, 449.3032).

N-(*N*-Benzoyl-L-phenylalanyl)-L-phenylalanol (**3**): ^{1}H NMR (chloroform-*d*, 500 MHz): δ_H (ppm) 7.71 (2H, *d*, *J* = 7.2 Hz, H-3'', -7''), 7.52 (1H, *t*, *J* = 7.4 Hz, H-5''), 7.43 (2H, *t*, *J* = 7.6 Hz, H-4'', -6''), 7.34-7.08 (10H, *m*, H-5,5' → 9,9'), 5.97 (1H, *d*, *J* = 8.0 Hz, 1-NHCO), 6.80 (1H, *d*, *J* = 7.6 Hz, 1''-NHCO), 4.78 (1H, *ddd*, *J* = 8.7, 7.6, 5.9 Hz, H-2), 4.09 (1H, *qt*, *J* = 7.7, 4.1 Hz, H-2'), 3.44 (2H, *m*, H-1'), 3.26 (1H, *dd*, *J* = 13.5, 5.9 Hz, H-3a), 3.05 (1H, *dd*, *J* = 13.5, 8.7 Hz, H-3b), 2.78 (1H, *dd*, *J* = 13.7, 7.5 Hz, H-3'a), 2.69 (1H, *dd*, *J* = 13.7, 7.3 Hz, H-3'b), 1.98 (1H, *t*, *J* = 5.9 Hz, 1'-OH). ^{13}C NMR (chloroform-*d*, 125 MHz): δ_C (ppm) 170.9 (C-1), 167.3 (C-1''), 137.4 (C-4), 136.9 (C-4'), 133.8 (C-2''), 132.0 (C-5''), 129.5 (C-6', -8'), 129.3 (C-6, -8), 129.0 (C-4'', -6''), 128.8 (C-5, -9), 128.7 (C-5', -9'), 127.4 (C-7'), 127.2 (C-3'', -7''), 126.8 (C-7), 63.7 (C-1'), 55.4 (C-2), 53.0 (C-2'), 38.8 (C-3), 37.0 (C-3'). HRESIMS *m/z* 425.1836 $[M+Na]^+$ (calcd for $C_{25}H_{26}N_2O_3Na$, 425.1841).

Moupinamide (**4**): ^{1}H NMR (acetone-d_6, 500 MHz): δ_H (ppm) 8.17 (1H, *s*, 7-OH), 7.97 (1H, *s*, 6'-OH), 7.44 (1H, *d*, *J* = 15.6 Hz, H-3), 7.16 (1H, *d*, *J* = 1.9 Hz, H-5), 7.06 (2H, *d*, *J* = 8.5 Hz, H-5', -7'), 7.04 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-9), 6.83 (1H, *d*, *J* = 8.1 Hz, H-8), 6.76 (2H, *d*, *J* = 8.5 Hz, H-4', -8'), 6.49 (1H, *d*, *J* = 15.6 Hz, H-2), 3.88 (3H, *s*, -OCH_3), 3.49 (2H, *m*, H-1'), 2.75 (2H, *t*, *J* = 7.4 Hz, H-2'). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C (ppm) 166.4 (C-1), 156.7 (C-6'), 149.1 (C-6), 148.6 (C-7), 140.3 (C-3), 131.2 (C-3'), 130.5 (C-5', -7'), 128.4 C-4), 122.6 (C-9), 120.1 (C-2), 116.1 (C-8, -4', -8'), 111.3 (C-5), 56.3 (-OCH_3), 41.9 (C-1'), 35.8 (C-2'). HRESIMS *m/z* 336.1207 $[M+Na]^+$ (calcd for $C_{18}H_{19}NO_4Na$, 336.1212).

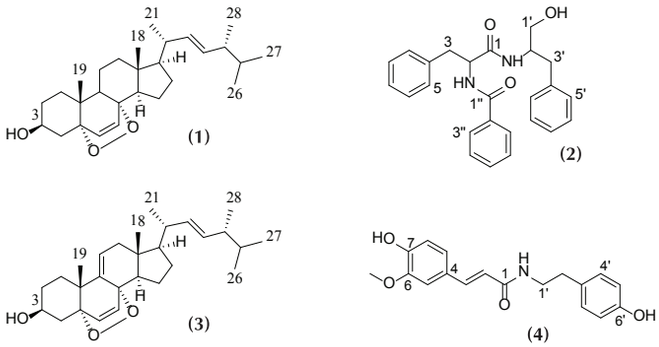

Fig. 1. The chemical structures of compounds 1-4.

Results and discussion

Compound **1** was isolated as a white solid. The ^{1}H NMR spectrum exhibited the signals for two *cis*-olefinic protons [δ_H 6.49 (1H, *d*, *J* = 8.5 Hz, H-6) and 6.23 (1H, *d*, *J* = 8.5 Hz, H-7)], two *trans*-olefinic protons [δ_H 5.28 (1H, *dd*, *J* = 7.5, 15.3 Hz, H-23)] and 5.21 (1H, *dd*, *J* = 8.2, 15.3 Hz, H-24)], one oxymethine proton (δ_H 3.76, H-3), and six methyl groups (δ_H 0.84-1.03). The ^{13}C NMR spectrum illustrated the presence of 28 carbons, including the signals for four olefinic carbons (δ_C 130.0-137.0), three oxygenated carbons (δ_C 66.5-82.5), six methyl carbons, and other aliphatic carbons. All above NMR data suggested that the structure of **1** was the ergostan-type steroid. These data closely resembled those of ergosterol peroxide [2]. Therefore, the structure of **1** was established as indicated.

Compound **2** was isolated as a white solid. The ^{1}H NMR spectrum displayed the closely similar signals relative to that of compound **1**, aside from the presence of an additional olefinic signal at δ_H 5.46 (1H, *dd*, *J* = 6.0, 2.0 Hz, H-11). The ^{13}C NMR spectrum of **2** exhibited 28 resonance signals, including the presence of two olefinic carbons [δ_C 116.0 (C-9), 119.5 (C-11)] instead of the C-9 methine and C-11 methylene signals in **1**. These data were also characteristic of the ergostan-type steroid and closely resembled those of 9.11-dehydroergosterol peroxide [4]. Therefore, the structure of **2** was concluded.

Compound **3** was isolated as a white solid. The ^{1}H NMR spectrum of **3** exhibited the signals of 15 aromatic protons (δ_H 7.00-7.80 ppm), one methine proton [δ_H 4.78 (1H, *ddd*, *J* = 8.7, 7.6, 5.9 Hz, H-2)] coupled with one methylene group [δ_H 3.26 (1H, *dd*, *J* = 13.5, 5.9 Hz, H-3a), 3.05 (1H, *dd*, *J* = 13.5, 8.7 Hz, H-3b)], one nitrogenated methine [δ_H 4.09 (1H, *qt*, *J* = 7.7, 4.1 Hz, H-2')], one methylene [δ_H 2.78 (1H, *dd*, *J* = 13.7, 7.5 Hz, H-3'a) and 2.69 (1H, *dd*, *J* = 13.7, 7.3 Hz, H-3'b)], and one hydroxymethyl [δ_H 3.44 (2H, *m*, H-1')]. Its ^{13}C NMR spectrum indicated the occurrence of 18 aromatic carbons (δ_C 125.0-136.9), two carbonyl carbons (δ_C 170.9 and 167.3), one hydroxymethyl (δ_C 63.7), two methylene (δ_C 38.8 and 37.0), and two nitrogenated carbons (δ_C 55.4 and 53.0). By comparing these data with those reported for *N*-(*N*-benzoyl-L-phenylalanyl)-L-phenylalanol [5], the structure of **3** was concluded as illustrated.

Compound **4** was isolated as a white solid. The ^{1}H NMR spectrum of **4** exhibited the signals for two *trans*-olefinic protons [δ_H 7.44 (1H, *d*, *J* = 15.6 Hz, H-3) and 6.49 (1H, *d*, *J* = 15.6 Hz, H-2)], one methoxy group [δ_H 3.88 (3H, *s*)], and two methylene groups [δ_H 3.49 (2H, *m*, H-1') and 2.75 (2H, *t*, *J* = 7.4 Hz, H-2')]. The signals of one pair of *ortho*-coupled protons [δ_H 7.06 (2H, *d*, *J* = 8.5 Hz, H-5', -7') and 6.76 (2H, *d*, *J* = 8.5 Hz, H-4', -8')] indicated the presence of 1.4-disubstituted benzene ring. Moreover, three aromatic protons of the 1,3,4-trisubstituted benzene ring was indicated [δ_H 6.83 (1H, *d*, *J* = 8.1 Hz, H-8), 7.04 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-9) and 7.16 (1H, *d*, *J* = 1.9 Hz, H-5)]. The ^{13}C NMR spectrum of **4** displayed resonances for one carbonyl (δ_C 166.4), 12 aromatic carbons (δ_C 95.4-167.2), two *trans*-olefinic carbons (δ_C 120.1, 140.3), one methoxy carbon (δ_C 56.3), and two methylene carbons (δ_C 35.8, 41.9). Therefore, the structure of **4** was determined as moupinamide, based on a comparison between these NMR data and the literature [6].

Conclusions

From the methanolic extract of *Solanum procumbens* (Solanaceae), four compounds were isolated, including ergosterol peroxide (**1**), 9.11-dehydroergosterol peroxide (**2**), *N*-(*N*-benzoyl-L-phenylalanyl)-L-phenylalanol (**3**), and moupinamide (**4**). The structures of isolated compounds were established based on NMR, MS data, and comparison with those in the literature. Moreover, all isolated compounds were first reported from *S. procumbens*.

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] V.C. Vo (1999), *Dictionary of Vietnamese Medicinal Plants*, Medical Publishing House, Hanoi, pp.152-153.

[2] S.M. Yu, H.J. Kim, E.R. Woo, H. Park (1994), "Some sesquiterpenoids and 5α,8α-epidioxysterols from *Solanu lyratum*", *Arch. Pharm. Res.*, **17(1)**, pp.1-4.

[3] T.T.P. Nguyen (2015), "Research on extraction and determination of chemical compounds from trunks and roots of *Solanum procumbens* Lour", *Proceeding of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS)*, pp.128-135.

[4] Y.K. Chen, Y.H. Kuo, B.H. Chiang, J.M. Lo, L.Y. Sheen (2009), "Cytotoxic activities of 9,11-dehydroergosterol peroxide and ergosterol peroxide from the fermentation mycellia of *Ganoderma lucidum* cultivated in the medium containing leguminous plants on Hep 3B cells", *J. Agric. Food Chem.*, **57**, pp.5713-5719.

[5] B. Xu, Z. Huang, C. Liu, Z. Cai, W. Pan, P. Cao, X. Hao, G. Liang (2009), "Synthesis and anti-hepatitis B virus activities of Matijing-Su derivatives", *Bioorg. Med. Chem.*, **17**, pp.3118-3125.

[6] J. Turnock, S. Cowan, A. Watson, B. Bartholomew, C. Bright, Z. Latif, S.D. Sarker, R.J. Nash (2001), "*N-trans*-feruloyltyramine from two species of the Solanaceae", *Biochem. Syst. Ecol.*, **29**, pp.209-211.