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Sterols from *Echinopsis oxygona (Link) zucc. ex Pfeiff*

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Echinopsis oxygona (Link) zucc. ex Pfeiff, commonly known as easter lily cactus and sea urchin cactus, is grown as an ornamental plant in the Philippines. Chemical investigation of the dichloromethane extracts of *E. oxygona* has led to the isolation of ostreasterol (**1**), ostreasteryl fatty acid esters (**2**) and ergosterol peroxide (**3**) from the flowers and a mixture of β-sitosterol (**4**) and stigmasterol (**5**) in about 2:1 ratio from the stems. Compounds **3**-**5** were reported to exhibit anticancer properties. This is the first report on the chemical constituents of *E. oxygona*.

Keywords: *Echinopsis oxygona (Link) zucc. ex Pfeiff*, Cactaceae, Ostreasterol, Ergosterol peroxide, β-Sitosterol, Stigmasterol.

INTRODUCTION

Echinopsis oxygona (Link) zucc. ex Pfeiff of the family Cactaceae is commonly known as easter lily cactus and sea urchin cactus [1]. It grows on rocks and walls at high altitudes in the Mountain Province and is cultivated as an ornamental plant at lower altitudes in the Philippines. This cactus has no report regarding the chemical constituents and biological activities. However, *Echinopsis tubiflora* (Pfeiffer) Zuccarini, a congener of *E. oxygona* was reported to contain 24-methylcholesterol (33.1% of total) and sitosterol (66.9% of total) which is of relevance to present report [2].

Herein, an isolation of ostreasterol (**1**), ostreasteryl fatty acid esters (**2**) and ergosterol peroxide (**3**) from the flowers and a mixture of β-sitosterol (**4**) and stigmasterol (**5**) in about 2:1 ratio from the stems is reported. This is the first report on the isolation of **1**-**5** (Fig. 1) from *E. oxygona.*

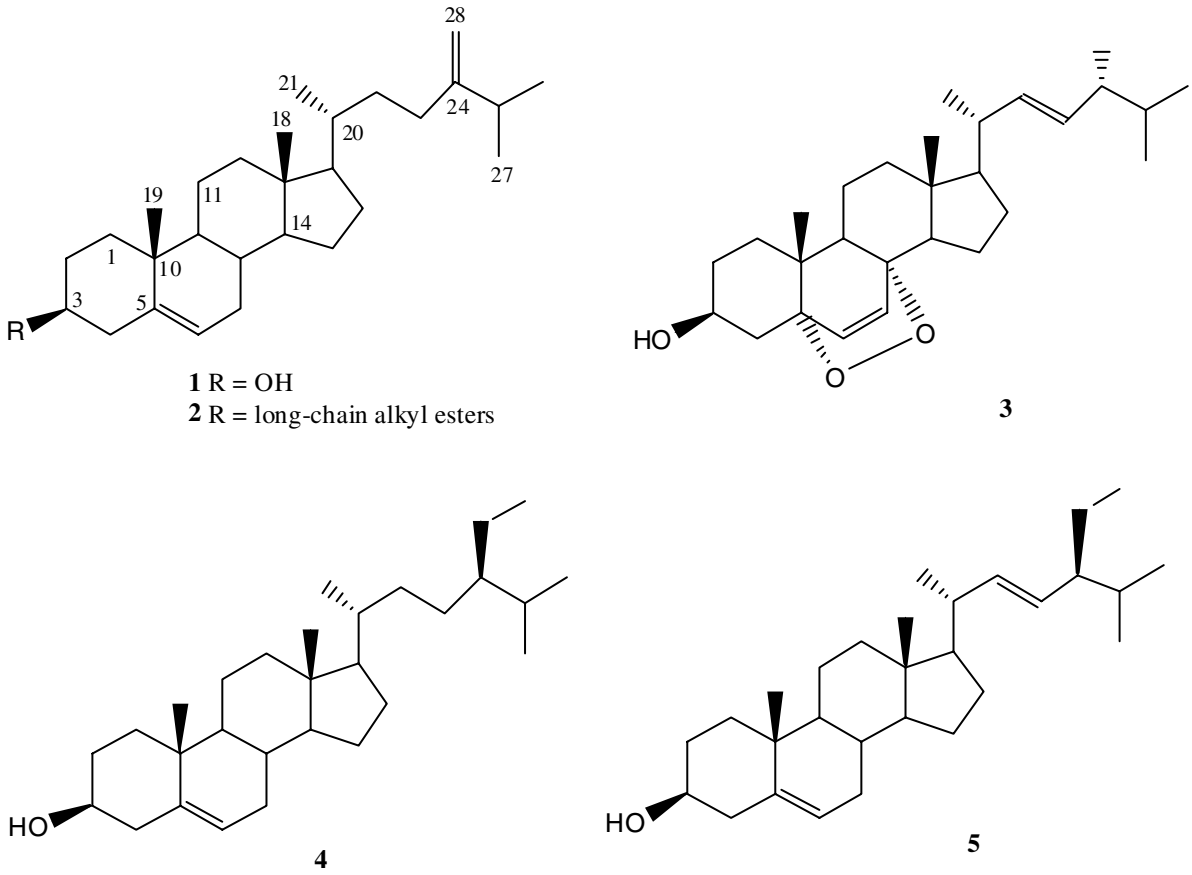
EXPERIMENTAL

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Spectra were referenced to the residual proton in $CDCl_3$ at δ 7.24 (^1H) and $CDCl_3$ at δ 77.0 (^{13}C). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (0.2 mm layer thickness) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample collection: The sample was collected from Lupao, Nueva Ecija, Philippines on January 15, 2018. It was authenticated as *Echinopsis oxygona (Link) zucc. ex Pfeiff* at the Biological Sciences Department, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines.

General isolation procedure: Initial chromatographic steps were performed using a glass column 12 inches in height and 0.5 inch internal; 5 mL fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns; 1 mL fractions were collected.

Isolation of the chemical constituents of *E. oxygona* flowers: The freeze-dried flowers (12.3 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.2808 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10 % increments. The



1 R = OH
2 R = long-chain alkyl esters

3

4

5

Fig. 1. Chemical structures of ostreasterol (**1**), ostreasteryl fatty acid esters (**2**), ergosterol peroxide (**3**), β-sitosterol (**4**) and stigmasterol (**5**) from *E. oxygona*

CH_2Cl_2 and 10% acetone in CH_2Cl_2 fractions were combined and rechromatographed by gradient elution using 5% EtOAc in petroleum ether, followed by 10% EtOAc in petroleum ether and finally 15% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum ether were combined and rechromatographed using 5% EtOAc in petroleum ether to yield **2** (1.8 mg). The fractions eluted with 10% and 15% EtOAc in petroleum ether were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **1** (3.3 mg) after washing with petroleum ether. The 20% to 40% acetone in CH_2Cl_2 fractions were combined and rechromatographed using 20% EtOAc in petroleum ether to yield **3** (1.2 mg) after washing with petroleum ether.

Isolation of the chemical constituents of *E. oxygona* stems: The freeze-dried stems (20.3 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.2611 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10 % increments. The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether to afford a mixture of **4** and **5** (2.1 mg) after washing with petroleum ether.

Ostreasterol (1): Colourless solid; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.50 (1H, m, H-3), 5.33 (1H, dd, *J* = 4.8, 2.4 Hz, H-6), 4.69 (1H, br s, H-28b), 4.64 (1H, d, *J* = 1.2 Hz, H-28a), 1.01 (3H, d, *J* = 7.2 Hz, H-26/H-27), 1.00 (3H, d, *J* = 6.6 Hz, H-26/H-27), 0.99 (3H, s, H-19), 0.93 (3H, d, *J* = 6.6 Hz, H-21), 0.66 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 37.24 (C-1), 30.96 (C-2), 71.80 (C-3), 42.28 (C-4), 140.74 (C-5), 121.71 (C-6), 31.90 (C-7), 31.90 (C-8), 50.11 (C-9), 36.49 (C-10), 21.07 (C-11), 39.77 (C-12), 42.35 (C-13), 56.75 (C-14), 24.28 (C-15), 28.20 (C-16), 55.98 (C-17), 11.85 (C-18), 19.39 (C-19), 35.74 (C-20), 18.70 (C-21), 34.68 (C-22), 30.96 (C-23), 156.88 (C-24), 33.79 (C-25), 21.86 (C-26), 21.99 (C-27), 105.92 (C-28).

Ostreasteryl fatty acid esters (2): Colourless solid; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.58 (1H, m, H-3), 1.35 (1H, d, *J* = 4.8 Hz, H-6), 4.69 (1H, br s, H-28b), 4.64 (1H, d, *J* = 1.2 Hz, H-28a), 1.01 (3H, d, *J* = 7.2 Hz, H-26/H-27), 1.00 (3H, d, *J* = 6.6 Hz, H-26/H-27), 1.00 (3H, s, H-19), 0.93 (3H, d, *J* = 6.6 Hz, H-21), 0.66 (3H, s, H-18), 2.26 (2H, t, *J* = 7.8 Hz, α-CH_2), 1.22-1.29 (br s, $(CH_2)_n$), 0.86 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 37.00 (C-1), 30.97 (C-2), 73.67 (C-3), 42.35 (C-4), 139.73 (C-5), 122.56 (C-6), 31.89 (C-7), 31.89 (C-8), 50.01 (C-9), 36.60 (C-10), 21.02 (C-11), 39.73 (C-12), 42.35 (C-13), 56.68 (C-14), 24.27 (C-15), 28.21 (C-16), 55.97 (C-17), 11.83 (C-18), 19.32 (C-19), 35.75 (C-20), 18.70 (C-21), 34.67 (C-22), 30.97 (C-23), 156.89 (C-24), 33.80 (C-25), 21.89 (C-26), 22.00 (C-27), 105.92 (C-28), 173.35 (C-1'), 34.73 (C-2'), 31.92 (C-3'), 22.69, 25.07, 29.11-29.70 (CH_2)n, 14.11 (CH_3).

Ergosterol peroxide (3): Colourless solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.48 (1H, d, *J* = 8.4 Hz, H-6), 6.23 (1H, d, *J*

= 8.4 Hz, H-7), 5.12 (1H, dd, *J* = 8.4, 15 Hz, H-22), 5.20 (1H, dd, *J* = 7.8, 15 Hz, H-23), 3.95 (1H, m, H-3), 0.80 (3H, s, H-18), 0.87 (3H, s, H-19), 0.98 (3H, d, *J* = 6.6 Hz, H-21), 0.80 (3H, d, *J* = 6.6 Hz, H-26), 0.82 (3H, d, *J* = 7.2 Hz, H-27), 0.89 (3H, d, *J* = 6.6 Hz, H-28); ^{13}C NMR (150 MHz, $CDCl_3$): δ 34.68 (C-1), 30.11 (C-2), 66.47 (C-3), 36.89, 36.92 (C-4, C-10), 82.14 (C-5), 135.40 (C-6), 130.74 (C-7), 79.41 (C-8), 51.68 (C-9), 20.87 (C-11), 39.34 (C-12), 44.56 (C-13), 51.08 (C-14), 23.39 (C-15), 28.64 (C-16), 56.19 (C-17), 12.86 (C-18), 18.17 (C-19), 39.72 (C-20), 19.63 (C-21), 135.19 (C-22), 132.30 (C-23), 42.77 (C-24), 33.06 (C-25), 19.94 (C-26), 20.62 (C-27), 17.55 (C-28).

β-Sitosterol (4): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (1H, m, H-3), 5.33 (1H, br d, *J* = 4.8 Hz, H-6), 0.66 (3H, s, H-18), 0.99 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.31 (C-4), 140.74 (C-5), 121.72 (C-6), 31.90 (C-7), 31.90 (C-8), 50.15 (C-9), 36.50 (C-10), 21.08 (C-11), 39.77 (C-12), 42.29 (C-13), 56.76 (C-14), 24.36 (C-15), 28.24 (C-16), 56.04 (C-17), 11.97 (C-18), 19.39 (C-19), 36.14 (C-20), 18.77 (C-21), 33.94 (C-22), 26.06 (C-23), 45.83 (C-24), 29.14 (C-25), 19.02 (C-26), 19.81 (C-27), 23.06 (C-28), 11.85 (C-29).

Stigmasterol (5): Colourless solid; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (1H, m, H-3), 5.35 (1H, br d, *J* = 4.8 Hz, H-6), 0.68 (3H, s, H-18), 0.99 (3H, s, H-19), 5.12 (1H, dd, *J* = 8.4, 15.0 Hz, H-22), 5.00 (1H, dd, *J* = 8.4, 15.0 Hz, H-23); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.29 (C-4), 140.74 (C-5), 121.72 (C-6), 31.90 (C-7), 31.90 (C-8), 50.15 (C-9), 36.50 (C-10), 21.08 (C-11), 39.67 (C-12), 42.29 (C-13), 56.76 (C-14), 24.36 (C-15), 28.91 (C-16), 55.94 (C-17), 12.04 (C-18), 19.39 (C-19), 40.49 (C-20), 21.08 (C-21), 138.31 (C-22), 129.26 (C-23), 51.23 (C-24), 31.91 (C-25), 21.21 (C-26), 18.97 (C-27), 25.40 (C-28), 12.25 (C-29).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *Echinopsis oxygona (Link) zucc. ex Pfeiff*, yielded ostreasterol (**1**), ostreasteryl fatty acid esters (**2**) and ergosterol peroxide (**3**) from the flowers and a mixture of β-sitosterol (**4**) and stigmasterol (**5**) in about 2:1 ratio from the stems. The NMR data of **1** are in accordance with those reported in the literature for ostreasterol [3]; **3** for ergosterol peroxide [4]; **4** for β-sitosterol [5] and **5** for stigmasterol [5]. Compound **1** is also called 24-methylenecholesterol and chalinasterol.

The chemical structure of **2** was deduced from **1** as follows. The oxymethine proton at δ 3.50 bonded to the carbon at δ 71.80 in **1** were deshielded to δ 4.58 and 73.67, respectively in **2**. These indicated that the hydroxyl in **1** was replaced by an alkyl ester in **2**. Additional resonances in the ^{1}H NMR spectrum of **2** were attributed to the long-chain alkyl esters at δ 1.22-1.29 (br s) for the long chain methylenes, δ 2.26 (t) for the α-methylene and δ 0.86 (t) for the terminal methyl [6]. In the ^{13}C NMR spectrum, additional resonances were assigned to the long-chain alkyl esters at δ 173.35 for the carboxylate, δ 34.73 for the α-methylene, δ 31.92 for the β-methylene, δ 22.69, 25.07, 29.11-29.70 for the long-chain methylenes and δ 14.11 for the terminal methyl [7].

The 2:1 ratio of the mixture of **4** and **5** was deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **4** at δ 5.33 and **5** at δ 5.33, 5.13 and 5.00 [5]. This ratio was supported by the relative intensities of the methyl singlets of **4** at δ 0.66 and **5** at δ 0.68 [5].

Although no biological activity tests were conducted on the isolated compounds, literature search revealed that **3-5** exhibited anticancer properties. Sterol **3** suppressed inflammatory response in RAW 264.7 macrophages and growth of HT29 colon adenocarcinoma cells [8]. It was shown to exhibit antitumor activity in multiple myeloma U266 cells, Walker carcinosarcoma, human mammary adenocarcinoma, human gastric tumor (SNU-1), human hepatoma (SUN-354), human colorectal tumor (SUN-C4) and murine sarcoma-180 cell lines [9]. It exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU- 145) human prostate cancer cells at μM concentrations [10] and suppressed cell growth and STAT1 mediated inflammatory responses in HT29 cells [11]. It inhibited the growth and induced apoptosis of HL60 human leukaemia cells at a concentration of 25 μM, inhibited TPA induced inflammation and tumor promotion in mice and suppressed proliferation of mouse and human lymphocytes stimulated with mitogens [12]. It showed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295 [13] and induced death of miR-378 cell [14]. On the other hand, β-sitosterol (**4**) inhibited the growth of human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [15]; showed to be effective for the treatment of benign prostatic hyperplasia [16]; and attenuated β-catenin and PCNA expression and quenched radical *in vitro* in colon carcinogenesis [17]. It also induced apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [18]. Furthermore, stigmasterol (**5**) showed therapeutic efficacy against *Ehrlich ascites* carcinoma bearing mice and cytostatic activity against Hep-2 and McCoy cells [19] and markedly inhibited tumour promotion [20].

Conclusion

The dichloromethane extracts of the flowers of *Echinopsis oxygona (Link) zucc. ex Pfeiff*, yielded ostreasterol (**1**), ostreasteryl fatty acid esters (**2**) and ergosterol peroxide (**3**), while the stems afforded β-sitosterol (**4**) and stigmasterol (**5**). This is the first report on the isolation of sterols **1**-**5** from this cactus.

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CONFLICT OF INTEREST

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

REFERENCES

1. M.D. Cheek and N.R. Crouch, *Bothalia*, **45**, 1953 (2015); https://doi.org/10.4102/abc.v45i1.1953
2. T.A. Salt, J.E. Tocker and J.H. Adler, *Phytochemistry*, **26**, 731 (1987); https://doi.org/10.1016/S0031-9422(00)84774-3
3. P. Permeh, S. Saeidnia, A. Mashinchian-Moradi and A.R. Gohari, *Nat. Prod. Res.*, **26**, 774 (2012); https://doi.org/10.1080/14786419.2010.548812
4. C.Y. Ragasa, V.D. Ebajo Jr., R.G. Reyes, R. Brkljaèa and S. Urban, *Der Pharm. Chem.*, **7**, 331 (2015).
5. C.Y. Ragasa, G.S. Lorena, E.H. Mandia, D.D. Raga and C.-C. Shen, *Am. J. Essent. Oils Nat. Prod.*, **1**, 7 (2013).
6. V.A.S. Ng, E.M.G. Agoo, C.-C. Shen and C.Y. Ragasa, *J. Appl. Pharm. Sci.*, **5(Suppl 1)**, 12 (2015).
7. C.Y. Ragasa, J.L. Caro and C.-C. Shen, *J. Appl. Pharm. Sci.*, **4**, 7 (2014).
8. M. Kobori, M. Yoshida, M. Ohnishi-Kameyama and H. Shinmoto, *Br. J. Pharmacol.*, **150**, 209 (2007); https://doi.org/10.1038/sj.bjp.0706972
9. Y.-H. Rhee, S.-J. Jeong, H.-J. Lee, H.-J. Lee, W. Koh, J.H. Jung, S.-H. Kim and K. Sung-Hoon, *BMC Cancer*, **12**, 28 (2012); https://doi.org/10.1186/1471-2407-12-28
10. Y.K. Chen, Y.H. Kuo, Y. Chiang, J.M. Lo and L.Y. Sheen, *J. Agric. Food Chem.*, **57**, 5713 (2009); https://doi.org/10.1021/jf900581h
11. A. Russo, V. Cardile, M. Piovano, S. Caggia, C.L. Espinoza and J.A. Garbarino, *Chem. Biol. Interact.*, **184**, 352 (2010); https://doi.org/10.1016/j.cbi.2010.01.032
12. F. Leon, L. Brouard, F. Torres, J. Quintana, A. Rivera, F. Estévez and J. Bermejo, *Chem. Biodivers.*, **5**, 120 (2008); https://doi.org/10.1002/cbdv.200890002
13. X. Liu, C.Y. Wang, C.L. Shao, X.Y. Wei, B.G. Wang, L.-L. Sun, C.-J. Zheng and H.-S. Guan, *Biochem. Syst. Ecol.*, **37**, 127 (2009); https://doi.org/10.1016/j.bse.2009.01.009
14. Q.P. Wu, Y.Z. Xie, Z. Deng, X.M. Li, W. Yang, C.-W. Jiao, L. Fang, S.-Z. Li, H.-H. Pan, A.J. Yee, D.Y. Lee, C. Li, Z. Zhang, J. Guo and B.B. Yang, *PLoS One*, **7**, e44579 (2012); https://doi.org/10.1371/journal.pone.0044579
15. A.B. Awad, M. Chinnam, C.S. Fink and P.G. Bradford, *Phytomed.*, **14**, 747 (2007); https://doi.org/10.1016/j.phymed.2007.01.003
16. G.K. Jayaprakasha, K.K. Mandadi, S.M. Poulose, Y. Jadegoud, G.A. Nagana Gowda and B.S. Patil, *Bioorg. Med. Chem.*, **15**, 4923 (2007); https://doi.org/10.1016/j.bmc.2007.04.044
17. A.A. Baskar, S. Ignacimuthu, G.M. Paulraj and K.S. Al Numair, *BMC Comp. Alternat. Med.*, **10**, 24 (2010); https://doi.org/10.1186/1472-6882-10-24
18. D.-O. Moon, K.-J. Lee, Y.H. Choi and G.-Y. Kim, *Int. Immunopharmacol.*, **7**, 1044 (2007); https://doi.org/10.1016/j.intimp.2007.03.010
19. M.A. Gómez, M.D. García and M.T. Sáenz, *Phytother. Res.*, **15**, 633 (2001); https://doi.org/10.1002/ptr.837
20. Y. Kasahara, K. Kumaki, S. Katagiri, K. Yasukawa, S. Yamanouchi, M. Takido, T. Akihisa and T. Tamura, *Phytother. Res.*, **8**, 327 (1994); https://doi.org/10.1002/ptr.2650080603