A Triterpene and a Depside from Parmotrema austrocetratum Elix and J. Johnst.

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ABSTRACT
Introduction: Parmotrema austrocetratum Elix and J. Johnst. (syn. Rimelia austrocetrata Elix and J. Johnst.) which belongs to a large genus of lichenized fungi, Parmotrema Massalongo under family Parmeliaceae was investigated for its chemical constituents. Methods: The compounds were isolated by silica gel chromatography and their chemical structures were elucidated by NMR spectroscopy. Results: Chemical investigation of the dichloromethane extract of Parmotrema austrocetratum Elix and J. Johnst. has led to the isolation of zeorin (1) and atranorin (2). Conclusion: P. austrocetratum shares similar chemical characteristic with other Parmotrema species which afforded atranorin. This work highlights the first reported isolation of 1 from P. austrocetratum and the genus Parmotrema. Key words: Parmotrema austrocetratum, Rimelia austrocetrata, Parmeliaceae, Zeorin, Atranorin.

INTRODUCTION
Parmotrema austrocetratum Elix and J. Johnst. (syn. Rimelia austrocetrata Elix and J. Johnst.) belongs to a large genus of lichenized fungi under family Parmeliaceae. It is a species belonging to Parmotrema austrocetratum. The genus Parmotrema refers to the perforate apothecia (Greek parmos = cup and trema = perforation). In the Philippines, P. austrocetratum is distributed in Northern Cordillera in Luzon island and Mount Apo in Mindanao island. The Philippine specimen chosen for our chemical investigation was gathered from the trunk of a Benguet pine (Pinus kesiya Royle ex Gordon) in Camp John Hay, Baguio City. Parmotrema austrocetratum was reported to contain atranorin and salazinic acid. Of relevance to our present report are several studies on the genus Parmotrema which reported the presence of atranorin in P. arnoldii, P. crinitum, P. perlatum, P. stuppeum, P. crinitum, P. dilatatum, P. eclipatum, P. endosulphureum, P. erubescent, P. flavescens, P. flavomedullosum, P. gardneri, P. latissimum, P. eucoemosnetum, P. masonii, P. mellissi, P. neotropicum, P. permutilatum, P. robustum, P. rubifaciens, P. subarnoldii, P. subisidiossum, P. subtomentum, P. wrightii, P. sancti-angeli, P. simulans, P. sorediferum, P. soredialphilaticum, P. hydrium, P. praesorediosum, P. rampoddense, P. tinctorum, P. reticulatum, P. negrosorientalum, P. lichexanthonicum, P. cetratum, P. crisiferum, P. defectum, P. grayanum, P. margaritatum, P. perlatum, P. pseudocrinitum, P. reticulatum, P. subtomentum.

We report herein the isolation of zeorin (1) and atranorin (2) (Figure 1) from P. austrocetratum. To the best of our knowledge this is the first report on the isolation of 1 from P. austrocetratum and the genus Parmotrema.

MATERIALS AND METHODS
General Experimental Procedure
NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl3 at 600 MHz for 1H NMR and 150 MHz for 13C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F254 and the plates were visualized by spraying with vanillin/H2SO4 solution followed by warming.

Sample Collection
The Philippine specimen chosen for our chemical investigation was gathered from the trunk of a Benguet pine (Pinus kesiya Royle ex Gordon) in Camp John Hay, Baguio City (date of collection: 14 October 2017).

Isolation of the Chemical Constituents of P. austrocetratum
The freeze-dried P. austrocetratum (17.52 g) was ground in a blender, soaked in CHCl3, for three days and then filtered. The filtrate was concentrated under vacuo. The crude extract (2.0 g) was subjected to silica gel column chromatography using petrol-ethyl acetate as eluent and the fractions were analyzed by TLC, and the active fractions were then subjected to HPLC purification using ethyl acetate as mobile phase and UV detector (254 nm). The purified fraction (10 mg) was dissolved in CDCl3 and then analyzed by 1H NMR and 13C NMR spectroscopy.

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vacuum to afford a crude extract (0.1578 g) which was chromatographed by gradient elution using petroleum ether, 2.5% EtOAc in petroleum ether, 5% EtOAc in petroleum ether, 7.5% EtOAc in petroleum ether, 10% EtOAc in petroleum ether, 15% EtOAc in petroleum ether, 15% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether, followed by CH$_2$Cl$_2$:CH$_3$CO$_2$H (1:1:8, v/v), CH$_2$Cl$_2$:CH$_3$CO$_2$H (15.1 mg) after washing with petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to afford 1 (4.3 mg) after washing with petroleum ether. The fractions eluted with CH$_2$Cl$_2$:CH$_3$CO$_2$H (0.5:0.5:9, v/v) were combined and rechromatographed using the same solvent to yield 2 (15.1 mg) after washing with petroleum ether.

**Zeorin** (1): $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 0.74 (s, CH$_3$-28), 0.85 (s, CH$_3$-25), 0.96 (s, CH$_3$-27), 1.00 (s, CH$_3$-26), 1.02 (s, CH$_3$-23), 1.13 (s, CH$_3$-24), 1.16, 1.19 (s, CH$_3$-29, CH$_3$-30), 3.94 (dt, $J$ = 4.2, 10.8 Hz) -13C NMR (150 MHz, CDCl$_3$): $\delta$ 40.33 (C-1), 18.50 (C-2), 43.79 (C-3), 33.60 (C-4), 61.07 (C-5), 69.30 (C-6), 102.82 (C-7), 108.53 (C-8), 169.07 (C-9), 39.33 (C-10), 21.03 (C-11), 23.98 (C-12), 49.77 (C-13), 41.86 (C-14), 34.32 (C-15), 21.90 (C-16), 53.94 (C-17), 43.99 (C-18), 41.21 (C-19), 26.58 (C-20), 51.05 (C-21), 73.90 (C-22), 36.73 (C-23), 22.10 (C-24), 17.11 (C-25), 18.25 (C-26), 17.05 (C-27), 16.07 (C-28), 28.75 (C-29), 30.87 (C-30).

**Atranorin** (2): $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 6.38 (s, H-5), 10.34 (s, H-8), 2.67 (s, CH$_3$-9), 6.50 (s, H-6'), 2.07 (s, CH$_3$-8'), 2.53 (s, CH$_3$-9'), 3.97 (s, OCH$_3$), 12.48 (s, 2-OH), 12.53 (s, 4-OH), 11.92 (s, 3'-OH); $^13$C NMR (150 MHz, CDCl$_3$): $\delta$ 102.82 (C-1), 169.07 (C-2), 108.53 (C-3), 167.47 (C-4), 112.84 (C-5), 152.42 (C-6), 169.68 (C-7), 193.82 (C-8), 25.56 (C-9), 151.97 (C-1'), 116.77 (C-2'), 162.86 (C-3'), 110.24 (C-4'), 139.85 (C-5'), 116.00 (C-6'), 172.18 (C-7'), 9.35 (C-8'), 24.01 (C-9'), 52.32 (OCH$_3$).

**RESULTS AND DISCUSSION**

Silica gel chromatography of the dichloromethane extract of *P. austrocetratum* has led to the isolation of zeorin (1) and atranorin (2). The structures of 1 and 2 were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their NMR data with literature data.

Although there is no reported biological activity for *P. austrocetratum*, the compounds isolated from the plant were reported to possess diverse activities. Zeorin (1) and atranorin (2) have shown antiangiogenic and antioxidant activities. Triterpene 1 also showed strong activity against bacteria and fungi. Depside 2 exhibited anti-proliferative action against malignant cell lines, $^{15}$ antioxidant effects,$^{16-19}$ and antibiotic action against *M. aurum.*$^{20}$ It was found to inhibit leukotriene B$_4$ synthesis in leukocytes, which might affect inflammatory processes$^{21}$ and modulates the wound healing process.$^{22}$

**CONCLUSION**

*P. austrocetratum* shares similar chemical characteristic with other *Parmotrema* species which yielded atranorin. This study highlights the first reported isolation of 1 from *P. austrocetratum* and the genus *Parmotrema.*

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

CH$_2$Cl$_2$: Dichloromethane; CH$_3$CN: Acetonitrile; EtOAc: Ethyl acetate; Et$_2$O: Diethyl ether.

**SUMMARY**

Chemical investigation of the dichloromethane extract of *Parmotrema austrocetratum* Elix and J. Johnst. has led to the isolation of a triterpene, zeorin (1) and a depside, atranorin (2). The structures of 1 and 2 were elucidated by 1D and 2D NMR spectroscopy and confirmed by comparison of their NMR data with literature data.

**REFERENCES**


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