STUDY ON CHEMICAL CONSTITUENTS OF THE LICHEN 
PARMOTREMA TINCTORUM (NYL.) HALE

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Abstract. A phytochemical investigation was conducted on foli ose lichen, Parmotrema tinctorum (Nyl.) Hale, collected in Lam Dong province, Vietnam. Color reactions for identification of lichen substances (+K deep yellow, +C red, +KC red, + P pale yellow) suggested the presence of atranorin, lecanoric acid, quinones, depsides, and xanthones containing two free hydroxyl groups in meta-position. In fact, six compounds, including atranol (1), methyl haematomate (2), divaricatinic acid (3), methyl divaricatinate (4), atranorin (5) and lecanoric acid (6) were isolated in the acetone extract, in which isolates (3) and (4) were reported for the first time in such species. Their structures were elucidated by X-ray diffraction or spectroscopic data and compared with those in references.

Keywords: atranorin, lecanoric acid, lichen, Parmotrema tinctorum, X-ray diffraction.

Classification numbers: 1.1.1; 1.1.6

1. INTRODUCTION

Lichens are symbiotic products of a mycobiont (fungal partner) and photobiont (algal partner) and are known to produce a range of unique secondary metabolites [1]. Characteristic compounds of lichens are depsides, depsidones, diphenyl ethers, benzofuran, usnic acid, and anthraquinone derivatives, presumably of fungal origin and their biological activities remain largely underexplored. However, a few have been shown to possess antibiotic, antmycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic and anti-proliferative activities [2, 3]. Parmotrema is a large genus in the Parmeliaceae with approximately 350 species of foliose lichens and a high level of diversity in the tropical areas of the world. In Vietnam, investigation on chemical constituents of Parmotrema has not been noticed so far as only few studies have paid attention on it, especially Parmotrema tinctorum (Nyl.) Hale. Previous studies
on its chemical constituents reported the presence of flavoxanthin, β-citraurin, atranorin, lecanorol, lecanoric acid, isolecanoric acid, salazinic acid, and some monocyclic aromatic compounds such as orsellinic acid, ethyl orsellinate and methyl β-orsellinate [4-10]. Moreover, P. tinctorum extracts showed presence of carbohydrates, phenols, flavanoids, tannins, terpenoids, coumarins and saponins which may be basis of its biological effects [11]. In fact, the extracts of P. tinctorum exhibited significant antioxidant, antibacterial, antifungal activities as well as inhibitory potential against carbohydrate digestive enzymes and aldose reductase [12-15]. Our preliminary study on cytotoxic activity of the P. tinctorum extracts showed that the acetone extract (at a concentration of 100 µg/mL) inhibited more than 50 % of MCF-7 and NCI-H460 cancer cell lines [9]. As part of our studies on bioactive secondary metabolites, we continue to show the isolation and identification of six compounds from the acetone extract of P. tinctorum collected in Lam Dong province, Vietnam. Spot tests on upper cortex with useful lichen reagents (K, C, KC, and P) were also conducted to suggest general identification of lichen substances.

2. MATERIALS AND METHODS

2.1. Lichen material

Parmotrema tinctorum (Nyl.) Hale was collected in Lam Dong province, Vietnam on December 2015. The scientific name was identified by Dr. Kawinnat Buurang (Lichen herbarium of Ramkhamhaeng University, Department of Biology, Faculty of Science, Ramkhamhaeng University, Thailand). A voucher specimen (No Par-0117) was deposited in the herbarium of the Department of Chemistry, Faculty of Science, Can Tho University of Medicine and Pharmacy, Can Tho City, Viet Nam.

2.2. General experimental procedures

The NMR experiments were performed on a Bruker DMX 300 and 500 spectrometers. HRMS-ESI was carried out on a MICROMASS ZABspecTOF spectrometer for electrospray ionization. Melting points were measured on a Melting Point Meter M5000 Krüss. The crystal data was collected on a Enraf-Nonius FR590-kappa diffractometer with a CCD area detector and graphite monochromated MoKα radiation. The structure was solved using direct methods, refined with the Shelx software package and expanded using Fourier techniques. Computing software programs for: Data Collection, Cell Refinement and Data Reduction: COLLECT/HKL2000. Structure solution: SHELX-S97. Structure Refinement: SHELXL2012; CRYSTALBUILDER. Molecular Graphics: ORTEP-III; MERCURY.

Spot tests were carried out with reagents K (10% KOH), C (30 % potassium hypochlorite) and K followed by C (KC), P (5 % p-phenylenediamine in ethanol). Column chromatography was performed on normal phase silica gel (40-63 µm, Kieselgel 60, Merck 7667). Thin layer chromatography (TLC) was performed on Kieselgel 60F254 plates (Merck) and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity ≥ 99.0 %.

2.3. Extraction and isolation

Air-dried crushed thallus of the lichen P. tinctorum (300 g) were successively and exhaustively extracted with 3 liters of acetone by a hot Soxhlet to give acetone extract (80.0 g,
\( \rho = 26.7\% \). When the acetone extract was evaporated under reduced pressure, a precipitate occurred and was filtered off (2.3 g). The precipitate after re-crystallized was subjected to a silica gel column chromatography and eluted by the solvent system of petroleum ether–ethyl acetate with increasing ethyl acetate ratios to obtain seven fractions from Ac1 to Ac7. The fraction Ac2 was subjected to preparative TLC using n-hexane–chloroform 8:2 as eluent to afford atranol (1, 3.5 mg) and methyl haematomate (2, 4.9 mg). The fraction Ac3 was subjected to silica gel re-chromatographed, eluting with n-hexane–ethyl acetate–acetic acid (95:5:0.5) to give methyl divaricatinate (4, 4.2 mg). The fraction Ac6 was subjected to a silica gel column and eluted with n-hexane: ethyl acetate (85:15) to yield divaricatinic acid (3, 5.4 mg) and lecanoric acid (6, 7.3 mg).

Atranol (1): yellow solid; M.p 124-125°C; \(^1H\) NMR (acetone-\(d_6\), 500 MHz) \( \delta_{H} \) : 10.69 (2H, s, 2-\(OH\), 4-\(OH\)), 10.27 (1H, s, H-7), 6.26 (2H, s, H-1, H-5), 2.23 (3H, s, H-8); \(^13C\) NMR (acetone-\(d_6\), 125 MHz) \( \delta_{C} \) : 108.4 (C-1), 163.0 (C-2), 109.2 (C-3), 163.0 (C-4), 108.4 (C-5), 151.5 (C-6), 194.1 (C-7), 22.2 (C-8); ESI-HRMS \( m/z \) 175.0373 [M+Na]\(^+\) (calcd. for C\(_8\)H\(_{12}\)O\(_3\)Na).

Methyl haematomate (2): white needles (acetone); M.p 146-147°C.

Divaricatinic acid (3): white needles (acetone).

Methyl divaricatinate (4): white solid; \(^1H\) NMR (CDCl\(_3\), 500 MHz) \( \delta_{H} \) : 11.69 (1H, s, 2-\(OH\)), 6.34 (1H, s, H-3), 6.29 (1H, s, H-5), 3.92 (3H, s, 7-OCH\(_3\)), 3.80 (3H, s, 4-OCH\(_3\)), 2.83 (2H, m, H-2'), 1.55 (2H, m, H-2'), 0.96 (3H, t, 7.5, H-3'); \(^13C\) NMR (CDCl\(_3\), 125 MHz) \( \delta_{C} \) : 104.7 (C-1), 165.5 (C-2), 98.8 (C-3), 163.9 (C-4), 110.7 (C-5), 147.7 (C-6), 171.9 (C-7), 55.2 (4-OCH\(_3\)), 51.8 (7-OCH\(_3\)), 38.9 (C-1'), 24.9 (C-2'), 14.2 (C-3').

Atranolin (5): colorless powder; \(^1H\) NMR (DMSO-\(d_6\), 500 MHz) \( \delta_{H} \) : 2.04 (3H, s, H-8'), 2.35 (3H, s, H-9'), 2.39 (3H, s, H-9''), 3.88 (3H, s, 7'-OCH\(_3\)), 6.41 (1H, s, H-5), 6.65 (1H, s, H-5'), 10.21 (1H, s, H-8), 10.52 (1H, s, 2-\(OH\)); \(^13C\) NMR (DMSO-\(d_6\), 125 MHz) \( \delta_{C} \) : 107.9 (C-1), 163.6 (C-2), 109.0 (C-3), 161.7 (C-4), 115.2 (C-5), 151.4 (C-6), 164.5 (C-7), 193.8 (C-8), 20.1 (C-9), 110.6 (C-1'), 157.4 (C-2'), 116.3 (C-3'), 148.8 (C-4'), 115.7 (C-5'), 136.5 (C-6'), 169.7 (C-7'), 9.3 (C-8'), 21.1 (C-9'), 52.3 (7'-OCH\(_3\)'); ESI-HRMS \( m/z \) 397.0890 [M+Na]\(^+\) (calcd. for C\(_{19}\)H\(_{25}\)O\(_8\)Na).

Lecanoric acid (6): pale yellow needles (acetone); \(^1H\) NMR (DMSO-\(d_6\), 300 MHz) \( \delta_{H} \) : 10.31 (1H, s, 2-\(OH\)), 9.99 (1H, s, 2'-\(OH\)), 6.62 (1H, d, 2.1, H-3'), 6.59 (1H, d, 2.1, H-5'), 6.22 (2H, s, H-3, H-5), 2.37 (3H, s, 8-CH\(_3\)), 2.35 (3H, s, 8'-CH\(_3\)); \(^13C\) NMR (DMSO-\(d_6\), 75 MHz) \( \delta_{C} \) : 108.1 (C-1), 160.0 (C-2), 100.4 (C-3), 161.0 (C-4), 109.8 (C-5), 139.4 (C-6), 166.6 (C-7), 21.37 (C-8), 116.3 (C-1'), 158.8 (C-2'), 107.3 (C-3'), 152.1 (C-4'), 114.6 (C-5'), 140.2 (C-6'), 170.4 (C-7'), 21.4 (C-8').

### 3. RESULTS AND DISCUSSION

Spot tests on upper cortex showed a deep yellow with K, pale yellow with P, red with C (Figure 1). The results suggested the presence of atranolin and related compounds due to a deep yellow color with K. A pale yellow with P should be involved in the occurrence of atranol, ethyl haematommate, and salazinic acid. The thallus gave red with C and red when +KC suggesting the presence of quinones, depsides and xanthones containing two free hydroxyl groups in meta-position [16]. Finally, by a rapid step analysis, color reactions gave useful hints for the presence of certain functional groups of a lichen substance and also for classification of lichens. However, color reactions on upper cortex can only provide general information of lichen substances. As a part of searching bioactive compounds from lichens, the extraction and isolation were then
performed to confirm the occurrence of the suggested compounds and also for evaluation
bioactivities of isolated compounds in the future.

![Image](image-url)  
*Figure 1. The result spot tests on the thallus P. tinctorum.*

Air-dried crushed thallus of *P. tinctorum* were extracted with acetone by a hot Soxhlet to
give acetone extract. Chromatographic purification of the acetone extract led to the isolation of
six compounds (1–6) (Figure 2).

![Chemical structures](image-url)  
*Figure 2. Structures of compounds 1–6 isolated from *P. tinctorum*.*

Compound 1 appeared as yellow solids and the ESI-HRMS showed an ion peak at \(m/z\)
175.0373 [M+Na]⁺ corresponding the molecular formula of C₈H₈O₄. The ¹H-NMR spectrum
exhibited six singlet protons for two chelated hydroxyl groups at δₓ 10.69 (2-OH, 4-OH), a
formyl proton at δₓ 10.27 (H-7), two aromatic protons at δₓ 6.26 (H-1, H-5) and a methyl group
at δₓ 2.23 (H-8). The ¹³C NMR spectrum showed eight carbon signals including a methyl group
[(δₓ 22.2 (C-8)], two aromatic methines δₓ 108.4 (C-1, C-5), a formyl group δₓ 194.1 (C-7), and
four quaternary aromatic carbon signals at δₓ 163.0, 163.0, 109.2 and 151.5. The obtained
spectroscopic data were suitable with the published ones [16]. Therefore compound 1 was
atranol.

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The structures of 2 and 3 were determined by X-ray diffraction as methyl haematomate and divaricatinic acid, respectively. CCDC 1811395 and 1811394 (compounds 2 and 3, respectively) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Compound 4 obtained as a white solid. The $^1$H-NMR spectrum displayed signals of one hydroxyphenyl group at $\delta_{H}$ 11.69 (1H, s, 2-OH ), two aromatic methine protons at $\delta_{H}$ 6.34 (1H, d, $J$ = 2.5 Hz, H-3) and 6.29 ppm (1H, d, $J$ = 2.5 Hz, H-5), two methoxy groups at $\delta_{H}$ 3.92 (3H, s, 7-OCH$_3$) and 3.80 ppm (3H, s, 4-OCH$_3$), one n-propyl group [ $\delta_{H}$ 2.83 (2H, m, H-1'), 1.55 (2H, m, H-2') and 0.96 (3H, t, $J$ = 7.5 Hz, H-3')]. The $^{13}$C-NMR spectrum showed the resonances of 12 carbons including one carbonyl ester group at $\delta_{C}$ 171.9 (C-7), two methoxy groups [ $\delta_{C}$ 55.2 (4-OCH$_3$) and 51.8 (7-OCH$_3$)], one n-propyl group [( $\delta_{C}$ 38.9 (C-1'), 24.9 (C-2') and 14.2 (C-3') ] and six aromatic carbons. Comparison with previously reported data [16] confirmed the structure of 4 as methyl divaricatinate.

Compound 5 was isolated as colorless powder. The $^1$H-NMR spectrum of 5 displayed one methoxy group at $\delta_{H}$ 3.88 (3H, s, 7'-OCH$_3$), one formyl group at $\delta_{H}$ 10.21 (1H, s, 8-CHO), three methyl groups [ $\delta_{H}$ 2.04 (3H, s, H-8'), 2.35 (3H, s, H-9) and 2.39 (3H, s, H-9')], two aromatic methine protons [ $\delta_{H}$ 6.41 (1H, s, H-5) and 6.65 (1H, s, H-5') and one chelated hydroxyl proton at $\delta_{H}$ 10.52 (1H, s, 2-OH)]. The $^{13}$C- and DEPT-NMR spectra displayed two carboxyl groups at $\delta_{C}$ 164.5 (C-7) and 169.7 (C-7'), an aldehyde carbon at $\delta_{C}$ 193.8 (C-8), a methoxy group at $\delta_{C}$ 52.3 (7'-OCH$_3$), three methyl groups [ $\delta_{C}$ 9.3 (C-8'), 20.1 (C-9) and 21.1 (C-9')] and twelve aromatic carbon signals. All these properties suggested that the structure of 5 was atranorin. These spectroscopic data were compatible with the published ones [9].

Structure of compound 6 was confirmed by the $^1$H-NMR spectrum as two chelated hydroxy groups at $\delta_{H}$ 10.31 (2-OH) and 9.99 (2'-OH), four aromatic methine protons [ $\delta_{H}$ 6.62 (1H, d, $J$ = 2.1 Hz, H-3'), 6.59 (1H, d, $J$ = 2.1 Hz, H-5') and 6.22 (2H, s, H-3, H-5), two methyl groups at $\delta_{H}$ 2.37 (8-CH$_3$) and 2.35 (8'-CH$_3$)]. The $^{13}$C NMR spectrum of 6 showed signals due to 16 carbons corresponding to two methyls, four aromatic methines, eight quaternary carbons and two carboxyl carbons. Comparison of these data with the ones in literature [16], suggested that compound 6 was lecanoric acid.
4. CONCLUSIONS

Spot tests on upper cortex of the lichen Parmotrema tinctorum (Nyl.) Hale in Vietnam suggested the presence of atranorin, lecanoric acid, quinones, depsides, and xanthones containing two free hydroxyl groups in meta-position. Actually, in present study, six compounds were isolated in the acetone extract, including atranol (1), methyl haematomate (2), divaricatinic acid (3), methyl divaricatinate (4), atranorin (5) and lecanoric acid (6). The compounds (3) and (4) were reported for the first time in such species. It would be of interest for further chemical investigations and evaluation cytotoxic effects of isolated compounds to discover a new source of bioactive substances from lichens in Vietnam.

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REFERENCES


