Two Aliphatic Acid Derivatives from the Cultured Mycobionts of *Lecanora nipponica*

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Spore-derived mycobionts of the lichen *Lecanora nipponica* were cultivated on a malt-yeast extract medium supplemented with 10% sucrose and their metabolites were investigated. Two new metabolites, methyl (2*Z*, 4*E*)-3-methoxycarbonyl-2-methyl-2,4-nonadienoate and (4*E*)-3-methoxycarbonyl-2-methyl-4-nonenoic acid were isolated. Their structures were determined by spectroscopic methods.

Key words: *Lecanora nipponica*, Lichen Mycobiont, Aliphatic Acid

Thalli of lichens of the genus *Lecanora* were known to contain a variety of characteristic lichen substances [1, 2]. Many depsides and depsidones were reported from the lichens of this genus such as *L. planaica* [3]. An aliphatic acidic compound, roccellic acid (**1**), its related lactonic acid, roccellaric acid (**2**), and a xanthone, thiophanic acid as well as depsides and depsidones were isolated from *L. rupicola* (L.) Zahlbr [4, 5]. On the other hand, the cultured mycobiont of *L. rupicola* produced neither depside nor depsidone, but novel chromones and roccellic acid [6]. A chemotype of the natural lichen *L. dispersa* contains 2,7-dichlorolichexanthone as the major product, but cultured spore isolates produced two typical lichen depsidones [7]. We have recently cultivated the mycobionts of *L. cinereocarnea* (= *L. leprosa*) and *L. iseana* and isolated from their cultures diverse novel dibenzofurans, which have never been isolated from the natural lichens [8, 9]. These studies demonstrated that cultures of mycobionts have an ability to produce lichen substances or novel metabolites, which could not be found in associated lichens [10]. From our interests in the metabolic capability of the mycobionts isolated from lichens of the genus *Lecanora*, we cultivated the spore-derived mycobionts of *Lecanora nipponica* H. Miyaw. and isolated two new acidic compounds **3** and **4** from their cultures. We report here the isolation and characterization of these compounds.

The polyspore-derived mycobionts of *L. nipponica* were cultivated on conventional malt-yeast extract medium supplemented with 10% sucrose at 18 °C in the dark. After cultivation over 15 months, the colonies were harvested and extracted with acetone. The extract was separated by a combination of preparative TLC to afford two new compounds **3** and **4**.

Compound **3** was obtained as colourless oil. Its HR-EIMS spectrum indicated a molecular formula of $C_{13}H_{20}O_4$. Its ^{1}H NMR spectrum exhibited signals for two methyl groups at $\delta = 0.90$ (t, $J = 7.0$ Hz) and 2.00 (s), three sets of methylene protons at $\delta =$ 1.32, 1.42 and 2.21, a pair of *trans* olefinic protons at $\delta = 5.95$ (dt, $J = 16.0$, 7.0 Hz) and 6.34 (dt, $J = 16.0$, 1.5 Hz) and two carbomethoxyl groups at $\delta = 3.75$ and 3.86 (each s). The ^{13}C NMR spectrum of **3** showed two methyls, two methoxyls, three methylenes, two sp^2 CH carbons and four sp^2 quaternary carbons, of which two were carbonyl carbons. ^{1}H-^{1}H COSY sequences starting from the methyl signal at $\delta = 0.90$ to the olefinic proton signals formulated a 1*E*-hexenyl residue. HMBC correlations from the olefinic proton (H-4) at $\delta = 6.34$ and methyl signal (H_3-10) at $\delta = 2.00$ to two sp^2 quaternary carbons indicated the hexenyl and methyl groups substituted at the tetra-substituted double bond where two carbomethoxyl groups were also substituted. Further HMBC cross peaks between H-4 and carbony carbon at $\delta = 169.6$ and between H_3-10 and carbony carbon at $\delta = 167.6$ as well as an important NOESY correlation between H-4 and H_3-10 depicted the substitution pattern at the double bond as shown. Thus, the isolated compound **3** was determined as methyl (2*Z*, 4*E*)-3-methoxycarbonyl-2-methyl-2,4-nonadienoate.

The MS spectrum of compound **4** established the composition $C_{12}H_{20}O_4$. The ^{1}H NMR spectral features of **4** were closely similar to those of **3**; the significant difference in their spectra being that **4** showed only one singlet for carbomethoxyl group and signals for two methine protons at $\delta = 2.68$ and 3.14,

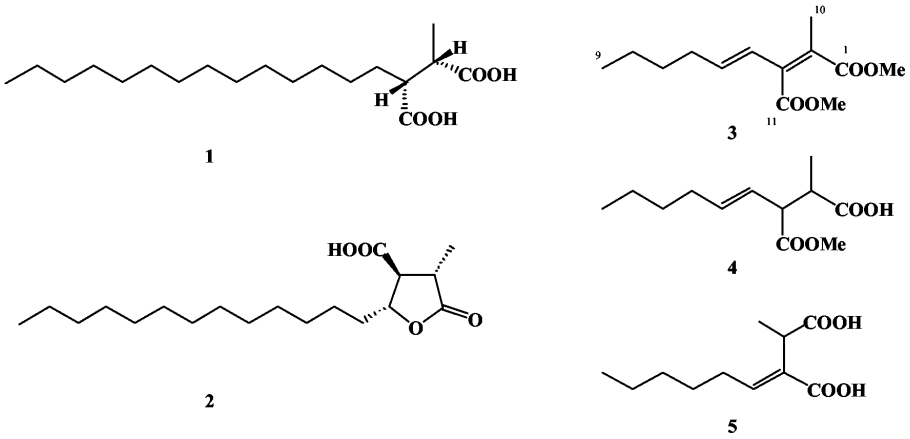

Fig. 1. Structures of roccellic acid (**1**), roccellaric acid (**2**), methyl (2*Z*, 4*E*)-3-methoxycarbonyl-2-methyl-2,4-nonadienoate (**3**), (4*E*)-3-methoxycarbonyl-2-methyl-4-nonen oic acid (**4**) and piliformic acid (**5**).

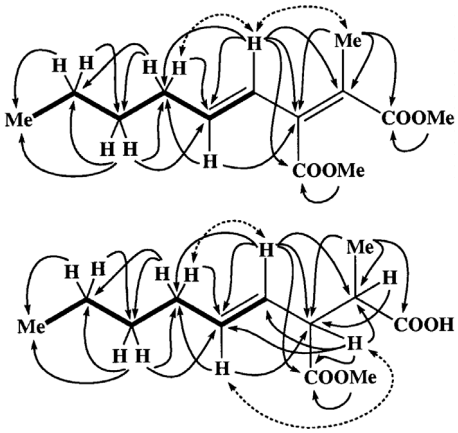

Fig. 2. ^{1}H-^{1}H COSY (bold lines), HMBC (bold arrows) and NOESY (dotted arrows) correlations observed for **3** and **4**.

which were not observed in **3**. Two sp^2 quaternary carbons were replaced by two sp^3 methine carbons in the ^{13}C NMR spectrum of **4**. These findings indicated **4** to be a demethylated compound of 2,3-dihydro derivative of **3**. The methoxycarbonyl group was placed at C-3 by its HMBC spectrum, which showed significant correlations from the methoxyl at $\delta = 3.63$ and a doublet at $\delta = 5.28$ to the carbonyl carbon at $\delta =$ 176.4 and from the methyl at $\delta = 1.10$ to the carbonyl carbon at $\delta = 178.0$. Accordingly, compound **4** was assigned to $(4E)$-3-methoxycarbonyl-2-methyl-4-nonenoic acid. The stereochemistry of C-3 and C-4 could not be determined due to a minute amount.

In the present study, we isolated two dicarboxylic acid derivatives from the cultured mycobionts of *L. nipponica*. It can be postulated that these compounds might most likely be biosynthesized from a C_8-fatty acid and a C_3-unit originated from oxaloacetate [11]. Analogous dicarboxylic acids and their related lactones represented by **1** and **2**, which possess a C_{14}-, C_{16}-, or C_{18}-chain as a fatty acid unit, have so far been isolated from natural lichens [1]. The dicarboxylic acids and their relatives with a C_8-fatty acid chain such as **3** and **4** had not been isolated from the lichens in nature, but piliformic acid (**5**) was reported from xylariaceous fungi *Xylaria mali* and *X. longipes* [12].

Experimental Section

Optical rotation was measured on a Jasco DIP-370 digital polarimeter. HR-EIMS were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with Varian VXR-500 spectrometer with tetramethylsilane as internal standard. Thin-layer chromatography was performed on precoated Kieselgel 60F_{254} plates (Merck) and spots were visualized under UV light.

Plant material. Specimens of *Lecanora nipponica* H. Miyaw. were collected from the bark of trees in Kimitsu, Boso Peninsula, Chiba Prefecture, Japan (200 m alt.). The voucher specimens were identified by Prof. H. Miyawaki, Saga University, Japan and were deposited at Osaka City Institute of Public Health and Environmental Sciences with the registration No. NH9730161. Mycobionts were obtained from the spores discharged from apothecia of a thallus, and were cultivated in test tubes containing modified MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g, H_2O 1 l, pH 7) at 18 °C in the dark. After cultivation for 15 months, the colonies and slants were harvested and freeze-dried.

Extraction and isolation. The harvested colonies (84 test tubes, freeze-dried weight 7.89 g) were extracted with acetone at r. t., and the combined extracts were concentrated under reduced pressure to give a residue (588 mg). The extract was repeatedly subjected to preparative TLC (toluene-acetone, 4:1 or toluene-AcOH, 9:1), giving rise to **3** (3.9 mg) and **4** (5.1 mg).

*Methyl (*2Z, 4E*)-3-methoxycarbonyl-2-methyl-2,4-nonadienoate (***3***).* Colorless oil. — ^{1}H NMR (499.99 MHz, $CDCl_3$): $\delta = 0.90$ (t, $J = 7.0$ Hz, 3H, H_3-9), 1.32 (br sext, $J = 8.0$ Hz, 2H, H_2-8), 1.42 (m, 2H, H_2-7), 2.00 (s, 3H, H_3-10), 2.21 (br q, $J = 7.0$ Hz, 2H, H_2-6), 3.75 (s, 3H, 1-OMe), 3.86 (s, 3H, 11-OMe), 5.95 (dt, $J = 16.0$, 7.0 Hz, 1H, H-5), 6.34 (dt, $J = 16.0$, 1.5 Hz, 1H, H-4). – ^{13}C{^{1}H} NMR (125.00 MHz, $CDCl_3$): $\delta = 13.5$ (C-10), 13.9 (C-9), 22.3 (C-8), 30.8 (C-7), 33.3 (C-6), 52.2 (1-OMe), 52.3 (11-OMe), 123.7 (C-4), 123.9 (C-2), 141.6 (C-5), 141.8 (C-3), 167.6 (C-1), 169.6 (C-11). – HR-EIMS *m/z*: calcd. for $C_{13}H_{20}O_4$ [M^+]: 240.1362; found 240.1354.

*(*4E*)-3-Methoxycarbonyl-2-methyl-4-nonenoic acid (***4***).* Colorless oil. – $[\alpha]_D^{22} - 102^\circ$ (c=0.5, MeOH). – ^{1}H NMR (499.99 MHz, CD_3OD): $\delta = 0.90$ (t, $J = 7.5$ Hz, 3H, H_3-9), 1.10 (br d, $J = 6.5$ Hz, 3H, H_3-10), 1.32 (m, 2H, H_2-8), 1.37 (m, 2H, H_2-7), 2.05 (br q, $J = 7.0$ Hz, 2H, H_2-6), 2.68 (m, 1H, H-2), 3.14 (br t, $J = 9.0$ Hz, 1H, H-3), 3.63 (s, 3H, 1-OMe), 5.28 (br dd, $J = 15.0$, 9.0 Hz, 1H, H-4), 5.65 (br dd, $J = 15.0$, 7.0 Hz, 1H, H-5). – ^{13}C{^{1}H} NMR (125.00 MHz, CD_3OD): $\delta = 14.2$ (C-9), 16.1 (C-10), 23.2 (C-8), 32.5 (C-7), 33.2 (C-6), 44.4 (C-2), 52.2 (11-OMe), 54.3 (C-3), 127.1 (C-4), 137.0 (C-5), 176.4 (C-11), 178.0 (C-1) – HR-EIMS *m/z*: calcd. for $C_{12}H_{20}O_4$ [M^+]: 228.1362; found 228.1351.

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