Two Aliphatic Acid Derivatives from the Cultured Mycobionts of Lecanora nipponica

Yukiko Takenaka\textsuperscript{a}, Nobuo Hamada\textsuperscript{b}, and Takao Tanahashi\textsuperscript{a}

\textsuperscript{a} Kobe Pharmaceutical University, 4-19-1, Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan
\textsuperscript{b} Osaka City Institute of Public Health and Environmental Sciences, 8-34, Tojo-cho, Tennouji-ku, Osaka 543-0026, Japan

Reprint requests to Prof. T. Tanahashi. Fax: +81-78-441-7546. E-mail: tanahashi@kobepharma-u.ac.jp

Z. Naturforsch. 60b, 1324 – 1326 (2005); received February 15, 2005

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, roccellaric acid (1), its related lactonic acid, roccelllic acid (2), and a xanthone, thiophanic acid as well as depsides and depsidone-related lactonic acid, roccellaric acid (2), as well as a xanthone, thiophanic acid as well as depsides and depsidone-related lactonic acid, roccellaric acid (2), were investigated. Two new metabolites, methyl (2\textsuperscript{E}, 4\textsuperscript{E})-3-methoxycarbonyl-2-methyl-2,4-nonadienoate and (4\textsuperscript{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

0932–0776 / 05 / 1200–1324 $ 06.00 c 2005 Verlag der Zeitschrift für Naturforschung, Tübingen · http://znaturforsch.com
Note 1325

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

Fig. 2. 1H-1H COSY (bold lines), HMBC (bold arrows) and NOESY (dotted arrows) correlations observed for 3 and 4, which were not observed in 3. Two sp² quaternary carbons were replaced by two sp³ methine carbons in the 13C 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 δ = 3.63 and a doublet at δ = 5.28 to the carbonyl carbon at δ = 176.4 and from the methyl at δ = 1.10 to the carbonyl carbon at δ = 178.0. Accordingly, compound 4 was assigned to (4E)-3-methoxycarbonyl-2-methyl-4-nonen oic 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 Lecanora nipponica. It can be postulated that these compounds might most likely be biosynthesized from a C₈-fatty acid and a C₃-unit originated from oxaloacetate [11]. Analogous dicarboxylic acids and their related lactones represented by 1 and 2, which possess a C₁₄-, C₁₆-, or C₁₈-chain as a fatty acid unit, have so far been isolated from natural lichens [1]. The dicarboxylic acids and their relatives with a C₈-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 Yasco DIP-370 digital polarimeter. HR-EIMS were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with a Varian VXR-500 spectrometer with tetramethylsilane as internal standard. Thin-layer chromatography was performed on precoated Kieselgel 60F₂₅₄ 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, H₂O 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. – 1H NMR (499.99 MHz, CDCl3): \( \delta = 0.90 \) (t, \( J = 7.0 \) Hz, 3H, H3-9), 1.32 (br sext, \( J = 8.0 \) Hz, 2H, H2-8), 1.42 (m, 2H, H2-7), 2.00 (s, 3H, H3-10), 2.21 (br q, \( J = 7.0 \) Hz, 2H, H2-6), 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). – 13C\{1H\} NMR (125.00 MHz, CDCl3): \( \delta = 13.5 \) (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 C13H20O4 [M+]: 240.1362; found 240.1354.

(4E,3)-3-Methoxycarbonyl-2-methyl-4-nonenoic acid (4). Colorless oil. – \([\alpha]_{D}^{22} = -102^\circ \) (c=0.5, MeOH). – 1H NMR (499.99 MHz, CD3OD): \( \delta = 0.90 \) (t, \( J = 7.5 \) Hz, 3H, H3-9), 1.10 (br d, \( J = 6.5 \) Hz, 3H, H3-10), 1.32 (m, 2H, H2-8), 1.37 (m, 2H, H2-7), 2.05 (br q, \( J = 7.0 \) Hz, 2H, H2-6), 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). – 13C\{1H\} NMR (125.00 MHz, CD3OD): \( \delta = 14.2 \) (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 C12H20O4 [M+]: 228.1362; found 228.1354.

Acknowledgements

This research was financially supported by Grant-in-Aid (C)(No.13836009 and 15510186) from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan, and the Kobe Pharmaceutical University Collaboration Fund. We are grateful to Dr. H. Miyawaki (Saga University, Japan) for identification of the voucher specimen. Thanks are also due to Dr. M. Sugiura (Kobe Pharmaceutical University) for 1H and 13C NMR spectra, and to Dr. K. Saiki (Kobe Pharmaceutical University) for mass spectra measurements.