# TMC-151 A Monoacetate, a New Polyketide from *Bionectria ochroleuca*

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A new polyketide, TMC-151 A monoacetate (**1**), along with a known analogue, TMC-151 F (**2**), was isolated from ethyl acetate extracts of the fermentation broth of *Bionectria ochroleuca* isolated in Taiwan. Their structures were elucidated on the basis of spectroscopic analysis. The antiproliferative activities of **1** and **2** were evaluated against MT-2 (human leukemia), A498 (human renal carcinoma), NPC-tw01 (human nasopharyngeal carcinoma), H-226 and A549 (non-small cell lung cancer) cell lines, and their $IC_{50}$ values ranged from 18.3 to 40.2 $\mu$M.

*Key words:* Hypocreales, *Bionectria ochroleuca*, Polyketides, Fermentation Broth, Cytotoxicity

## Introduction

Hypocrealean fungi are ecologically highly diverse. They are saprotrophic, necrotrophic or biotrophic, and many species are well known to have great economic importance [1]. *Bionectria ochroleuca* (Schwein.) Schroers & Samuels [anamorph: *Clonostachys rosea* (Link: Fr.) Schroers *et al.*] has been found to produce a variety of antibiotics and could be used as a biocontrol agent against plant pathogenic fungi [2]. Recently, the fermentation broth of *B. ochroleuca* was found to exhibit significant *anti*-proliferative activities against human Colo205, HL60 and Hela cancer cell lines in our preliminary screening of fungal extracts for cytotoxicity. These findings prompted us to investigate the cytotoxic agents from this fungus. Therefore, a series of bioassay-guided chemical examinations on the fermentation broth were carried out which resulted in the isolation of a new polyketide **1** together with the known analogue **2**. This paper describes the isolation and structure elucidation of **1** and **2** as well as their cytotoxicities.

## Results and Discussion

From the fermentation broth of *B. ochroleuca* two polyketides **1** and **2** were identified. These compounds were isolated and purified using repetitive Sephadex LH-20 column chromatography and HPLC by the guidance of cytotoxicity tests. Compound **2**, a major component, was obtained as a colorless powder whose spectral data were compatible with those of TMC-151 F, previously isolated from *Gliocladium catenulatum* Gilman & Abbott TC 1280 [3, 4].

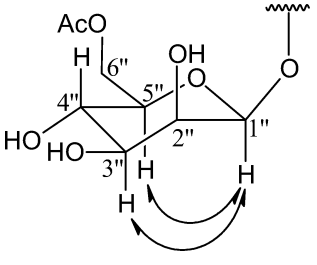


Fig. 1. Key NOESY correlations of 6″-*O*-acetyl-$\beta$-mannopyranoside in **1**.

Compound **1**, a colorless powder, has the molecular formula $C_{43}H_{76}O_{16}$ as deduced from its HRFABMS and $^{13}$C NMR spectral data. The IR spectrum of **1** indicated the presence of hydroxyl (3347 cm$^{-1}$) and carbonyl (1705 cm$^{-1}$) groups. The $^{13}$C NMR and DEPT spectra of **1** exhibited a $C_{43}$ skeleton closely related to that of TMC-151 A (**3**) [3], with the exception of two additional carbon signals at $\delta_C$ = 21.2 and 172.7 ascribable to an acetyl functionality. In the $^1$H NMR spectrum of **1**, the signals corresponding to the aliphatic chain (H-3 ∼ $H_3$-29), the mannitol moiety ($H_2$-1′ ∼ $H_2$-6′), and the glycopyranoside (H-1″ ∼ $H_2$-6″) were also closely related to those of **3**, except that the H-6″a and H-6″b signals were shifted from $\delta_H$ = 3.49 and 3.70 to lower fields ($\delta_H$ = 4.25 and 4.42, respectively). The glycopyranoside partial structure was further deduced to be a 6″-*O*-acetyl-$\beta$-mannopyranoside as evidenced from a large vici-

Table 1. $^{1}H$ and $^{13}C$ NMR data of **1** ($CD_3OD$, 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$).

| No. | $^{13}C$ | $^{1}H$ | COSY | HMBC (H→C) |
|---|---|---|---|---|
| 1 | 169.9 | | | |
| 2 | 128.7 | | | |
| 3 | 148.4 | 6.86 (1H, *J* = 1.3, 9.8 Hz) | 4 | 1, 5, 21 |
| 4 | 38.1 | 2.76 (1H, m) | 3, 5, 22 | 2, 3, 5, 22 |
| 5 | 83.6 | 3.83 (1H, d, *J* = 8.5 Hz) | 4 | 3, 4, 6, 7, 22, 23 |
| 6 | 137.2 | | | |
| 7 | 134.4 | 5.31 (1H, d, *J* = 9.2 Hz) | 8 | 5, 8, 23 |
| 8 | 37.0 | 2.60 (1H, m) | 7, 9, 24 | 6, 7, 9, 24 |
| 9 | 84.5 | 3.65 (1H, d, *J* = 9.5 Hz) | 8 | 7, 8, 10, 11, 24, 25 |
| 10 | 134.7 | | | |
| 11 | 135.0 | 5.52 (1H, d, *J* = 9.3 Hz) | 12 | 9, 13, 25 |
| 12 | 36.1 | 2.73 (1H, m) | 11, 13, 26 | 10, 11, 13, 26 |
| 13 | 87.4 | 3.43 (1H, m) | 12, 14 | 11, 12, 26, 27, 1″ |
| 14 | 34.3 | 1.87 (1H, m) | 13, 15, 27 | |
| 15a | 43.9 | 1.00 (1H, m) | 14, 15b | 13, 14, 16, 17, 27, 28 |
| 15b | 43.9 | 1.40 (1H, m) | 14, 15a | 13, 14, 16, 17, 27, 28 |
| 16 | 28.8 | 1.62 (1H, m) | 28 | 15, 17, 28 |
| 17a | 46.1 | 0.93 (1H, m) | 17b | 15, 16, 18, 28, 29 |
| 17b | 46.1 | 1.27 (1H, m) | 17a | 15, 16, 18, 28, 29 |
| 18 | 32.9 | 1.45 (1H, m) | 29 | |
| 19a | 29.9 | 1.12 (1H, m) | 19b, 20 | 17, 18, 20, 29 |
| 19b | 29.9 | 1.44 (1H, m) | 19a, 20 | 18 |
| 20 | 11.4 | 0.88 (3H, t, *J* = 6.4 Hz) | 19 | 18, 19 |
| 21 | 13.0 | 1.90 (3H, s) | | 1, 2, 3 |
| 22 | 16.8 | 0.86 (3H, d, *J* = 6.7 Hz) | 4 | 3, 4, 5 |
| 23 | 11.5 | 1.66 (3H, s) | | 5, 6, 7 |
| 24 | 17.8 | 0.75 (3H, d, *J* = 6.8 Hz) | 8 | 7, 8, 9 |
| 25 | 11.0 | 1.62 (3H, s) | | 9, 10, 11 |
| 26 | 18.7 | 0.97 (3H, d, *J* = 6.9 Hz) | 12 | 11, 12, 13 |
| 27 | 16.7 | 0.95 (3H, d, *J* = 6.8 Hz) | 14 | 13, 14, 15 |
| 28 | 21.0 | 0.90 (3H, d, *J* = 7.5 Hz) | 16 | 15, 16, 17 |
| 29 | 20.7 | 0.89 (3H, d, *J* = 6.4 Hz) | 18 | 17 |
| 1′a | 64.5 | 3.53 (1H, dd, *J* = 6.2, 11.5 Hz) | 1′b, 2′ | 2′, 3′ |
| 1′b | 64.5 | 3.64 (1H, m) | 1′a, 2′ | 2′ |
| 2′ | 72.0 | 3.97 (1H, m) | 1′, 3′ | |
| 3′ | 73.9 | 5.20 (1H, dd, *J* = 1.4, 7.7 Hz) | 2′ | 1, 1′, 2′ |
| 4′ | 71.3 | 3.95 (1H, dd, *J* = 1.4, 9.4 Hz) | 5′ | 2′, 3′, 5′ |
| 5′ | 72.3 | 3.43 (1H, m) | 4′, 6′a | |
| 6′a | 65.0 | 3.63 (1H, m) | 5′, 6′b | 5′ |
| 6′b | 65.0 | 3.80 (1H, dd, *J* = 3.0, 11.5 Hz) | 6′a | |
| 1″ | 102.7 | 4.45 (1H, br s) | | 13, 2″, 3″ |
| 2″ | 72.5 | 3.91 (1H, d, *J* = 3.1 Hz) | 3″ | 3″, 4″ |
| 3″ | 75.5 | 3.38 (1H, dd, *J* = 3.1, 9.6 Hz) | 2″, 4″ | 4″ |
| 4″ | 68.9 | 3.51 (1H, d, *J* = 9.6 Hz) | 3″, 5″ | 3″, 6″ |
| 5″ | 75.8 | 3.36 (1H, m) | 4″, 6″ | 6″ |
| 6″a | 65.5 | 4.25 (1H, dd, *J* = 7.2, 11.7 Hz) | 5″, 6″b | 5″, –O*C*OCH$_3$ |
| 6″b | 65.5 | 4.42 (1H, dd, *J* = 1.8, 11.7 Hz) | 5″, 6″a | 4″, –O*C*OCH$_3$ |
| –OCO*C*H$_3$ | 21.2 | 2.08 (3H, s) | | –O*C*OCH$_3$ |
| –O*C*OCH$_3$ | 172.7 | | | |

nal coupling constant between H-3″ and H-4″ ($^{3}J$ = 9.6 Hz) and a non-detectable coupling constant between H-1″ and H-2″, mutual n*O*e correlations between H-1″, H-3″ and H-5″ indicating the axial orientations of H-1″, -3″ and -5″ (Fig. 1), and the oxymethylene protons $H_2$-6″ at $\delta_H$ = 4.25 and 4.42 exhibiting a long range correlation with an acetyl carbonyl carbon signal at $\delta_C$ = 172.7 in the HMBC spectrum. The residual key connectivities of other fragments were also elucidated by the complete assignment of the HMBC spectrum where the anomeric H-1″ signal at $\delta_H$ = 4.45 correlated with the oxygenated carbon C-13 resonance at $\delta_C$ = 87.4, and the mannitol H-3′ signal at $\delta_H$ = 5.20 interacted with the carbonyl carbon C-1 resonance at $\delta_C$ = 169.9. Further analysis of all the 2D NMR data allowed the complete assignment of the

Table 2. $IC_{50}$ values of **1** and **2** against five human cancer cell lines.

| Compounds | $IC_{50}$ ($\mu$M)[a] MT-2[b] | A498[c] | NPC-tw01[d] | H-226[e] | A549[f] |
|---|---|---|---|---|---|
| **1** | 40.2 | 29.3 | 23.9 | 31.3 | 37.9 |
| **2** | 37.2 | 24.3 | 18.3 | 25.9 | 32.6 |
| Doxorubicin[g] | 0.037 | 0.447 | 0.035 | 0.199 | 0.837 |

[a] Cells were treated with various concentrations of test compounds for 3 days. Cell growth was determined by MTT assay. The $IC_{50}$ value resulting from 50 % inhibition of cell growth was calculated. Each value represents the mean of three independent experiments; [b] MT-2 as human leukemia cell line; [c] A498 as human renal carcinoma cell line; [d] NPC-tw01 as human nasopharyngeal carcinoma cell line; [e] H-226 as human non-small cell lung cancer cell line; [f] A549 as human non-small cell lung cancer cell line; [g] Doxorubicin, the chemotherarpeutic drug, as a reference compound in this study.

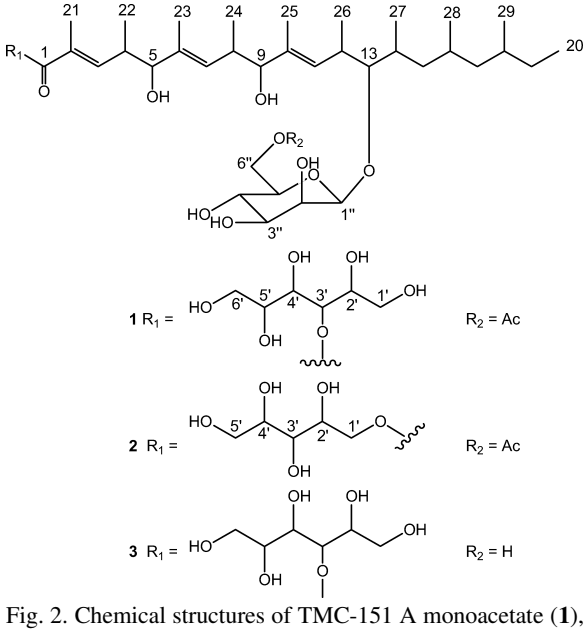


Fig. 2. Chemical structures of TMC-151 A monoacetate (**1**), TMC-151 F (**2**), and TMC-151 A (**3**).

$^1$H and $^{13}$C NMR spectra of **1**, as illustrated in Table 1. Accordingly, compound **1** was concluded to be the 6″-*O*-acetyl analogue of TMC-151 A, and named as TMC-151 A monoacetate as shown in Fig. 2.

This is the first report on polyketide antibiotics from *B. ochroleuca* in Taiwan. In this report we demonstrated a new structure accompanied by a known one. However, there are still many other minor analogues in this fungal strain that remain to be accumulated and identified.

Compounds **1** and **2** were further evaluated for their cytotoxicities against MT-2 (human leukemia), A498 (human renal carcinoma), NPC-tw01 (human nasopharyngeal carcinoma), H-226 and A549 (non-small cell lung cancer) cells. The cell viabilities were assessed through the MTT assay. As shown in Table 2, **1** and **2** with $IC_{50}$ values ranging from 18.3 to 40.2 $\mu$M showed only minor toxicity toward five tested cancer cells when compared with the positive control doxorubicin.

## Experimental Section

*General*

Optical rotations were measured on a JASCO P-1020 digital polarimeter (Kyoto, Japan). $^1$H and $^{13}$C NMR spectra were acquired on a Bruker DMX-500 SB spectrometer (Ettlingen, Germany). Mass spectra were obtained using a Finnigan Thermo Quest MAT 95XL spectrometer (Bremen, Germany). IR spectra were recorded on a Thermo Mattson IR 300 spectrometer (Califonia, USA). UV spectra were measured on a Hitachi U-2800 spectrophotometer (Tokyo, Japan). Column chromatography was carried out with Sephadex LH-20 gel (Amersham Biosciences, Uppsala, Sweden). Pre-coated Si gel plates (Si 60 $F_{254}$, 0.2 mm, Merck, Darmstadt, Germany) were used for analytical TLC.

*Fermentation of bionectria ochroleuca*

*Bionectria ochroleuca* (strain No. 91111210) was inoculated into 250 mL Erlenmeyer flasks containing 2 g malt extract (Bacto, Sparks, USA) and 100 mL deionized water. The fermentation was conducted under static conditions at 25 °C for two weeks.

*Extraction and isolation*

The mycelia together with the fermentation broth from the above fermentation were extracted three times with EtOAc by stirring at r. t. for 1 h. The organic layer was separated and concentrated in vacuum to dryness (6.5 g). This residue was re-dissolved in 20 mL of MeOH and filtered, then applied onto a column of Sephadex LH-20 (3 × 65 cm) and eluted with MeOH (2.7 mL/min). Every 23 mL of eluent was collected as one fraction and each of these fractions was analyzed by thin layer chromatography, using a solution of EtOAc/AcOH/$H_2O$ (85 : 10 : 10) for development. UV 254 nm illumination and vanillin-sulfuric acid charring was used to group the compounds with similar skeleton. The bioactive fractions (fr. 11 ∼ 13), determined by antiproliferative tests of human Colo205, HL60 and Hela cancer cells, were combined and evaporated under reduced pressure to yield 1.5 g of the bionectide mixture. The bionectide mixture was further purified by repetitive HPLC on a Hypersil ODS semi-preparative column (10 × 250 mm, Thermo Electron Corp., Bellefonte, USA) with MeCN/$H_2O$ (7 : 3) as eluent to afford **1** (7.5 mg) and **2** (10.0 mg), retention time: 16.2 and 18.0 min, respectively.

*TMC-151 A monoacetate (**1**):* Colorless powder. – $[\alpha]_D^{20}$ = +2.1° (*c* = 0.1, MeOH). – UV/vis (MeOH): $\lambda_{max}$ (lg $\varepsilon$) = 208 nm (4.20). – IR (KBr): $\nu$ = 3347 (–OH), 2959, 2925, 2873, 1705 (C=O), 1652, 1455, 1372, 1230, 1074, 1021 $cm^{-1}$. – $^1H$ NMR data: see Table 1. – $^{13}C$ NMR data: see Table 1. – MS (FAB): *m/z* (%) = 871 $[M + Na]^+$. – MS (HRFAB): *m/z* (%) = 871.4998 (calcd. 871.5031 for $C_{43}H_{76}O_{16}Na$).

*TMC151-F (**2**):* Colorless powder. – $[\alpha]_D^{20}$ = −0.85° (*c* = 0.1, MeOH). – UV/vis (MeOH): $\lambda_{max}$ (lg $\varepsilon$) = 207 (4.25). – IR (KBr): $\nu$ = 3353 (−OH), 2960, 2873, 1705 (C=O), 1651, 1455, 1373, 1270, 1228, 1072, 1023 $cm^{-1}$. – MS (FAB): *m/z* (%) = 841 $[M + Na]^+$.

*Cell culture*

Human leukemia MT-2 cells and non-small cell lung cancer H-226 and A549 cells were maintained in RPMI-1640 medium supplied with 10 % fetal bovine serum, 100 units $mL^{-1}$ penicillin and 100 $\mu$g $mL^{-1}$ streptomycin. Human renal carcinoma A498 cells and nasopharyngeal carcinoma NPC-tw01 cells were maintained in MEM medium supplied with 10 % fetal bovine serum, 100 units $mL^{-1}$ penicillin and 100 $\mu$g $mL^{-1}$ streptomycin.

*Growth inhibition assay*

Cell growth in the presence or absence of experimental agents was determined using the MTT-microculture tetrazolium assay [5]. Briefly, 100 $\mu$L of cell suspension in logarithmic growth phase were seeded into a 96-well plate (MT-2, $1.5 \times 10^4$ per well; NPC-tw01, $2 \times 10^3$ per well; A498, H-226, and A549, $4 \times 10^3$ per well). After 24 h, the cells were exposed to various concentrations of the test compound in a volume of 50 $\mu$L for 72 h. Two hours prior to the end of incubation, 15 $\mu$L MTT solution (5 mg $mL^{-1}$) was added into the culture medium. Cells were lysised with 75 $\mu$L of MTT lysis buffer (20 % SDS-50 % DMF) and cell lysis solution were incubated at 37 °C for another 12 h to dissolve the dark blue crystals. The absorption of the formazan solution at 570 nm was measured using a microplate reader.

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