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Note

Novel Compounds from the Mycelia and Fruiting Bodies of *Climacodon septentrionalis*

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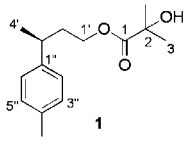
A novel ester (1) along with a known compound (2) were isolated from the mycelia of *Climacodon septentrionalis*. A novel furanone (3) and a known compound (4) were purified from the fruiting bodies of the fungus. The respective structures of 1 and 3 were determined as those of (*S*)-3-*p*-tolylbutyl 2-hydroxy-2-methylpropanoate and (−)-5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5*H*)-one by interpreting the spectroscopic data.

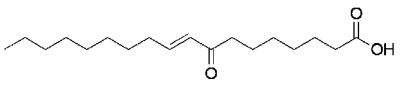
Key words: *Climacodon septentrionalis*; mycelium; fruiting body; structural determination

It is said that "plants act as producers, animals as consumers, and fungi as restorers and decomposers." In other words, plants create organic compounds by means of photosynthesis and animals consume such plants. Then fungi, including mushrooms, play an important role in restoring the plants and animals back to the land. There are some differences in the structures of metabolic products by fungi compared to those by plants and animals. Among various basidiomycetous mycelia and fruiting bodies, we paid attention to *Climacodon septentrionalis* (Japanese name, ezoharitake) and tried to isolate the constituents from the fungus, because only a report regarding a chemical study of sterols from the fruiting bodies of the fungus has so far been published[1)] and there is no data about the constituents of the mycelia. We report that our results succeeded in isolating and structurally determining two new compounds from the mycelia and the fruiting bodies of the fungi.

The compounds in the lyophilized mycelia of *C. septentrionalis* were successively extracted with *n*-hexane, EtOAc, and then EtOH, and the hexane-soluble part was fractionated by repeated chromatography. A novel compound (**1**) and a known one (**2**) were purified as results. In contrast, a new furanone (**3**) and a known one (**4**) were isolated from the EtOH extract of the fruiting bodies of the fungus.



1



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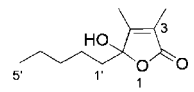

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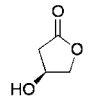

4

Compound **1** was purified as white crystals. Its molecular formula was determined as $C_{15}H_{22}O_3$ by HRESIMS, m/z 273.14699 $[M + Na]^+$ (calcd. for $C_{15}H_{22}O_3Na$, 273.14666), and the degree of unsaturation of the compound was five. The complete assignment of all the protons and the carbons was accomplished by DEPT, HMQC, COSY, and HMBC experiments (Table 1). The molecular formula, IR absorption at 1738 and 1558 cm^{-1}, and signals at δ_H 7.04 (2H, d, $J = 8.4$ Hz, H-2″ and H-6″), 7.09 (2H, d, $J = 8.4$ Hz, H-3″ and H-5″), and δ_C 126.7 (C-2″ and C-6″), 129.3 (C-3″ and C-5″), 135.7 (C-4″), 142.9 (C-1″), and 177.4 (C-1) in the ^{1}H- and ^{13}C-NMR data indicted this compound to be an ester possessing a *p*-substituted phenyl group. The HMBC correlations between H-3/C-2, H-3/C-1, H-3/C-2Me, H-2Me/C-2, H-2Me/C-3 and H-2Me/C-1, and the chemical shift of C-1 (δ_C 177.4) and C-2 (δ_C 71.9) indicated that the acid part of the compound was 2-hydroxy-2-methylpropanoic acid (Fig. 1). The presence of a *p*-tolyl moiety in the alcohol part was indicated by the COSY (H-2″, H-6″/H-3″, H-5″) and HMBC (H-2″, H-6″/C-3″, C-5″, H-2″, H-6″/C-4″, H-2″, H-6″/C-6″, C-2″, H-3″, H-5″/C-1″, H-3″, H-5″/C-4″Me, H-3″, H-5″/C-5″, C-3″, H-4″Me/C-3″, C-5″, and H-4″Me/C-4″) correlations (Fig. 1). The structure of the other part in the alcohol, *n*-butyl, and the position of the linkage of the butyl with the *p*-tolyl were determined by the COSY (H-1′/H-2′, H-2′/H-3′, H-3′/H-4′) and HMBC (H-1′/C-2′, H-1′/C-3′, H-2′/C-1′, H-2′/C-3′, H-2′/C-4′, H-3′/C-1′, H-3′/C-2′, H-3′/C-4′, H-3′/C-1″, H-3′/C-2″, C-6″, H-2′/C-1″, H-4′/C-3′, H-4′/C-2′, H-4′/C-1″, H-2″, H-6″/C-3′) cross peaks (Fig. 1). The HMBC correlation between H-1′/C-1 established the ester linkage. The absolute configuration of **1** was determined to be *S* by comparing its specific rotation, $[\alpha]_D = +26$ ($c = 0.10$, $CHCl_3$) with that {$[\alpha]_D = +30$ ($c = 1.0$, $CHCl_3$)} of the corresponding alcohol, (*S*)-3-(*p*-tolyl)butanol. Although neither of the enantiomers of the alcohol has been isolated from a natural source, they have both already been synthesized.[2)] The structure of **1** was therefore determined as (*S*)-3-*p*-tolylbutyl 2-hydroxy-2-methylpropanoate.

Compound **2** was identified as (*E*)-8-oxooctadec-9-enoic acid. This compound has been reported as an aldehyde dehydrogenase inhibitor from fruiting bodies of the fungus, *Clitocybe clavipes* (Japanese name,

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Table 1. ^{1}H- and ^{13}C-NMR Data for **1** (in $CDCl_3$)

Position	^{1}H δ (multiplicity, J in Hz)	^{13}C
1	—	177.4
2	—	71.9
2-CH_3	1.38 (3H, s)*	27.15*
3	1.39 (3H, s)*	27.18*
1′	4.04 (2H, m)	64.4
2′	1.91 (2H, m)	36.8
3′	2.78 (1H, m)	36.3
4′	1.25 (3H, d, 7.0)	22.4
1″	—	142.9
2″, 6″	7.04 (1H, d, 8.4)	126.7
3″, 5″	7.09 (1H, d, 8.4)	129.3
4″	—	135.7
4″-CH_3	2.30 (3H, s)	21.0

*interchangeable

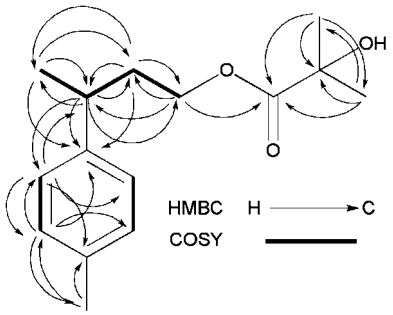

Fig. 1. COSY and HMBC Correlations for **1**.

hoteishimeji).[3] If ethanol is consumed with this mushroom, the person may experience one or more of the following symptoms: profound flushing, metallic taste, palpitations, hyperventilation, hypertension, tachycardia, nausea, vomiting, and occasionally collapse.

Compound **3** was isolated as a colorless oil. Its molecular formula was determined as $C_{11}H_{18}O_3$ by HRESIMS, m/z 221.1133 $[M+Na]^+$ (calcd. for $C_{11}H_{18}O_3Na$, 221.1154). The complete assignment of all the protons and carbons was accomplished by DEPT, HMQC, COSY, and HMBC experiments as follows: ^{1}H-NMR (in CD_3OD) δ: 0.86 (3H, t, $J = 6.4$ Hz, H-5′), 1.22–1.32 (6H, m, H-2′, 3′, 4′), 1.74 (1H, m, H-1′a), 1.80 (3H, br.s, H-3Me), 1.92 (3H, br.s, H-4Me), 1.95 (1H, m, H-1′b); ^{13}C-NMR δ: 8.4 (C-3Me), 10.6 (C-4Me), 13.9 (C-5′), 22.4, (C-4′), 22.6 (C-3′), 31.5 (C-2′), 36.0 (C-1′), 106.8 (C-5), 125.3 (C-3), 157.6 (C-4), 172.0 (C-2). All the data except for the specific rotation, $[\alpha]_D = +6.7$ ($c = 0.17$, EtOH), were identical with those of (−)-5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5*H*)-one, whose specific rotation, $[\alpha]_D = -6.7$ ($c = 0.30$, EtOH), and whose absolute configuration have not previously been determined.[4] Compound **3** is therefore novel, and its structure was determined as (+)-5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5*H*)-one. The absolute configuration of **3** remains unknown. The antipode of **3** has been isolated from stromata of *Epichloe typhina* and *Phleum pratense* as an antifungal compound against *Cladosporium herbarum*.[4] The corresponding compound, whose planar structure was the same as that of **3** but whose specific rotation was not measured, has also been purified from blue light-illuminated cress seedlings as a growth inhibitor against the hypocotyl growth of cress seedlings.[5]

Compound **4** was identified as (*S*)-3-hydroxy-γ-butyrolactone by comparing its spectroscopic data, including the specific rotation, with those of both enantiomers that are commercially available. Crude extracts of the mycelia and fruiting bodies of the fungus inhibited the growth of MRSA; although we tried to isolate the active principle(s), the activity disappeared during the isolation procedure and none of the compounds obtained therefore showed any activity.

Experimental

General experiments. ^{1}H-NMR spectra were recorded by a Jeol lambda-500 spectrometer at 500 MHz, while ^{13}C-NMR spectra were recorded on the same instrument at 125 MHz. The HRESIMS data were measured by a JMS-T100LC mass spectrometer. HPLC separation was performed with a Jasco Gulliver system. Silica gel plates (Merck F254) and silica gel 60 N (Merck 100–200 mesh) were respectively used for analytical TLC and for flash column chromatography.

Fungal materials and incubation. Mature fruiting bodies of *Climocodon septentrionalis* were collected from Narusawa village in Yamanashi Prefecture of Japan on September 2004. The strain was isolated from one of the fruiting bodies, and the isolate is deposited in the culture collection of Shizuoka University. The culture medium was potato dextrose broth (Difco) adjusted to pH 5.5. The liquid culture was carried out for 4 weeks in glass bottles at 25 °C while shaking.

Extraction and isolation (from mycelia). The wet mycelia of *C. septentrionalis* after cultivation were obtained by centrifugation ($7000 \times g$, 30 min) and lyophilized. The dried mycelia (60.3 g) were successively extracted with *n*-hexane (1.5 L, 3 times), EtOAc (1.5 L, 3 times), and then EtOH (1.5 L, 3 times). The residue (4.97 g) obtained after removing *n*-hexane was fractionated by silica gel flash column chromatography, eluting with solvents of *n*-hexane/EtOAc (9:1, 8:2, 6:4), CH_2Cl_2/acetone (8:2, 6:4), acetone, and MeOH to obtain sixteen fractions (fractions 1 to 16). Fraction 5 (1.4 g) was separated by silica gel flash column chromatography again with elution by *n*-hexane/EtOAc (6:4), CH_2Cl_2/MeOH (5:5), and MeOH to give five fractions (fractions 5-1 to 5-5). Fraction 5-3 was further separated by reversed-phase HPLC in an ODS column (Grand Pack ODS-A S-5 YC) with elution by 90% MeOH to afford compound **2** (0.5 mg). Fraction 6 (275 mg) was separated by reversed-phase MPLC in an ODS column (Ultra Pack ODS-S-50) with elution by MeOH to give six fractions (fractions 6-1 to 6-6). Fraction 6-3 was further separated by HPLC in a silica gel column (Senshu Pak Aquasil) with the elution by *n*-hexane/EtOAc (7:43) to afford compound **1** (1.3 mg).

Extraction and isolation (from fruiting bodies). The fresh fruiting bodies of *C. septentrionalis* (2.4 kg) were extracted with EtOH (4 L, three times). The resulting residue was then further extracted with acetone (10 L). The combined solution was concentrated under reduced pressure and partitioned between *n*-hexane and H_2O and then between EtOAc and H_2O. The hexane-soluble part (10.3 g) was fractionated by silica gel flash column chromatography (*n*-hexane/EtOAc 9:1, 7:3, 5:5; CH_2Cl_2/acetone 9:1, 5:5; and MeOH, 1.0 L each) to obtain thirteen fractions (fractions 1 to 13). Fraction 5 (1.523 g) was further separated by HPLC in a silica gel column (Senshu Pak Aquasil, *n*-hexane/EtOAc 6:4) to afford compound **4** (0.5 mg). The EtOAc-soluble part (3.2 g) was fractionated by MPLC in an ODS column (Ultra Pack ODS-S-50D, 95% MeOH) to give nine fractions. Fraction 3 was further separated by HPLC in a silica gel column (Senshu Pak Aquasil, *n*-hexane/EtOAc 5:5) to afford compound **3** (1.7 mg).

Acknowledgment

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