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Armillariols A to C from the culture broth of *Armillaria* sp.

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ABSTRACT

Three novel compounds were isolated from the culture broth of *Armillaria* sp. Their structures were elucidated mainly by spectroscopic data analyses. All the compounds regulated hypocotyl and root growth of lettuce.

Introduction

The genus *Armillaria* (English name, Honey Fungus; Japanese name, Naratake) belonging to the family physalacriaceae is a well known group of edible mushroom throughout the world. People have utilized it for its medicinal properties and edible qualities for a long time. On the other hand, the genus has been also known as a serious plant pathogen which causes root rot in various plant species[1] and the phenomenon is called 'Armillaria root disease'.[2,3] The rot is one of the most serious diseases of plants, which occurs in many broadleaf trees, conifers, and several herbaceous plants including *Alchemilla mollis, Beta vulgaris,* and so on.[4] Furthermore, it is known that the penetration of *Armillaria* mycelia into the fungi *Entoloma abortivum* and *Wynnea americana* induces spherical deformity of the fruiting bodies of those mushrooms.[5] These facts mean that *Armillaria* produces allelopathic substance(s). Protoilludane sesquiterpene aryl esters have been isolated from the genus *Armillaria* mushrooms and *Clitocybe illudens*.[6–12] Those compounds showed antimicrobial activity[7,13,14] and cytotoxicity against human cancer cell lines.[15,16] However, there are no evidences that the compounds are toxic principles for 'Armillaria root disease'. Therefore, we tried to isolate compounds with plant growth regulatory activity from the fungus.

Here, we describe the isolation, structural determination, and biological activity of three novel compounds from the culture broth of a strain of the genus.

Results and discussion

Isolation of the active compounds from *Armillaria* sp. was guided by their growth-regulating activity on lettuce. Since culture broth of the fungus inhibited the growth, the broth was extracted

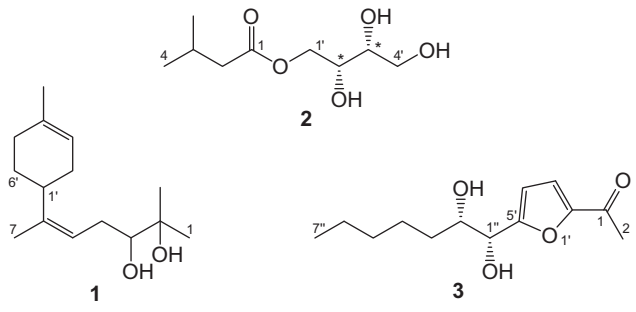

Figure 1. Structure of **1**–**3**.

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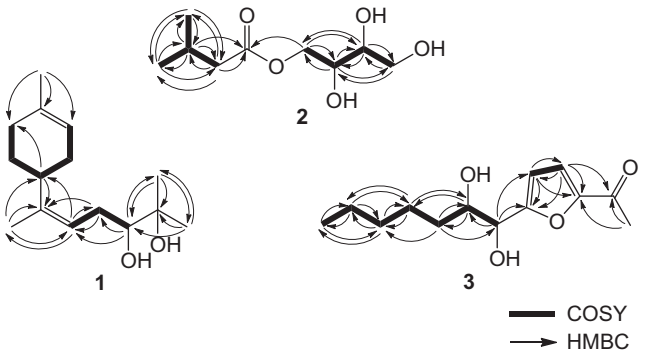

Figure 2. COSY and HMBC correlations in **1**–**3**.

with hexane, EtOAc, and 1-BuOH, successively. The active fractions, the hexane soluble-, and the EtOAc soluble-fractions, were subjected to repeated chromatography respectively to afford armillariol A to C (**1**–**3**) (Fig. 1).

Armillariol A (**1**) was purified as colorless oil. The molecular formula was determined as $C_{15}H_{26}O_2$ by HR-ESI-MS *m*/*z* 261.1826 $[M+Na]^+$ (calcd for $C_{15}H_{26}NaO_2$, 261.1830), indicating the presence of three degrees of unsaturation in the molecule. Structure of **1** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC (Fig. 2). The DEPT experiment indicated the presence of four methyls, four methylenes, four methines, and three quaternary carbons. The molecular formula, the unsaturation degrees, ^{13}C NMR data (δ_C 72.6, 77.9, 120.8, 120.9, 133.8, 143.5, Table 1), and the DEPT data indicated the presence of a ring, two double bonds, and two hydroxy groups in the molecule. The complete assignment of the protons and carbons of NMR was accomplished as shown in Table 1. The structure of the alkyl chain (C1–C7) was elucidated by the COSY correlations (H3/H4; H4/H5) and the HMBC correlations (H1/C2, C2-CH_3, C3; H2-CH_3/C1, C2, C3; H3/C2-CH_3, C4, C5; H4/C5, C6; H5/C4, C7; H7/C5, C6) (Fig. 2). The presence of the cyclohexene moiety (C1′–C6′) and its linking positions to a methyl and the chain were confirmed by the COSY correlations (H1′/H2′, H6′; H2′/H3′; H5′/H6′) and the HMBC correlations (H5/C1′, H7/C1′, H1′/C5′; H4′-CH_3 /C3′, C4′, C5′) (Fig. 2). NOE was observed between H4 and H1′ in the NOESY and NOE-difference experiments, indicating that the geometry of the double bond was *Z*. As a result, structure of **1** was supposed to be (*Z*)-2-methyl-6-(4-methylcyclohex-3-enyl)hept-5-ene-2,3-diol (Fig. 1). 2-Methyl-6-(4-methylcyclohex-3-en-1-yl)-hept-6-ene-2,3-diol (10,11-dihydroxy-10,11*H*-β-bisabolene) that has the same skeleton as **1** has been isolated from the aerial parts of the plant *Acritopappus confertus*.[17]

Armillariol B (**2**) was purified as pale yellow oil. Its molecular formula was determined as $C_9H_{18}O_5$ by HR-ESI-MS *m*/*z* 229.1066 $[M+Na]^+$ (calcd for $C_9H_{18}NaO_5$, 229.1052), indicating the presence of one degree of unsaturation in the molecule. Structure of **2** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC (Fig. 2). The one unsaturation degree was explained by the presence of an ester (δ_C 173.6). The acid part, 3-methylbutanoate, was elucidated by the COSY correlations (H2/H3; H3/H4, H3-CH_3) and the HMBC correlations (H2/C1, C3, C4, C3-CH_3; H3/C2, C4, C3-CH_3, H4/C2, C3, C3-CH_3, H3-CH_3/C2, C3, C4) (Fig. 2). The COSY correlations (H1′/H2′, H2′/H3′, H3′/H4′) and the HMBC correlations (H1′/C2′, C3′, C1; H2′/C1′, C3′, C4′; H3′/C1′, C2′; H4′/C2′, C3′) indicated the structure of the alcohol part and its linkage position to the acid part. As a consequence, **2** was determined as 2,3,4-trihydroxybutyl 3-methylbutanoate (Fig. 1).

Armillariol C (**3**) was purified as pale yellow oil. Its molecular formula was determined as $C_{13}H_{20}O_4$ by HR-ESI-MS *m*/*z* 263.1224 $[M+Na]^+$ (calcd for $C_{13}H_{20}NaO_4$, 263.1259), indicating that the unsaturation degree in the molecule was four. Structure of **1** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC (Fig. 2). The DEPT experiment indicated the presence of two methyls, four methylenes, four methines, and three quaternary carbons. The single carbon chain from C1″ to C7″ was constructed by the ^1H and ^{13}C NMR (Table 1) spectra along with COSY correlations (H3′/H4′, H1″/H2″, H2″/H3″, H3″/H4″, H4″/H5″, H6″/H7″) and HMBC ones (H2/C1, C2′; H3′/C-1, C2′, C4′, C5′; H4′/C2′, C3′, C5′; H1″/C4′, C5′, C2″; H2″/C3″, C4″; H3″/C1″, C2″, C4″, C5″; H4″/C2″, C5″, C6″; H5″/C4″, C6″, C7″; H6″/C4″, C5″, C7″; H7″/H5″, H6″). The molecular formula, the presence of a carbonyl-conjugated diene (C1, δ_C 185.5; C2′, δ_C 152.0; C3′, δ_C 118.6, δ_H 7.12 (d, *J* = 3.7 Hz); C4′, δ_C 109.8, δ_H 6.47 (d, *J* = 3.7 Hz); C5′, δ_C 159.6) including two quaternary sp^2 carbons, and the unsaturation degree indicated that this compound possessed a furan. As a result, structure of **3** was determined to be 1-(5-(1,2-dihydroxyheptyl)furan-2-yl)ethanone (Fig. 1).

Confirmation of the planar structures and determination of stereochemistry of **2** and **3** were performed by X-ray crystallography

Table 1
^1H and ^{13}C NMR data for **1–3** (**1** and **3** in $CDCl_3$, **2** in CD_3OD)

Position	1		Position	2		Position	3	
	^1H δ (mult., *J* in Hz)	^{13}C δ		^1H δ (mult., *J* in Hz)	^{13}C δ		^1H δ (mult., *J* in Hz)	^{13}C δ
1	1.22 (s)	26.6	1		174.8	1		186.5
2		72.6	2	2.22 (d, 7.0)	44.1	2	2.43 (s)	25.9
3	3.36 (dd, 8.9, 4.0)	77.9	3	2.08 (m)	26.8	2′		152.0
4	2.16 (m)	29.8	4	0.95 (d, 6.7)	22.7	3′	7.12 (d, 3.7)	118.6
	2.20 (m)		3-CH_3	0.95 (d, 6.7)	22.7	4′	6.47 (d, 3.7)	109.8
5	5.18 (dd, 7.3, 7.0)	120.9	1′	4.13 (m)	66.6	5′		159.6
6		143.5		4.16 (m)		1″	4.56 (br s)	71.1
7	1.66 (br. s)	19.5	2′	3.82 (m)	70.4	2″	3.92 (m)	73.2
1′	2.60 (m)	35.4	3′	3.60 (m)	73.0	3″	1.45 (m)	33.0
2′	1.80 (m)	29.4	4′	3.60 (m)	64.1		1.52 (m)	
	2.02 (m)					4″	1.28 (m)	25.2
3′	5.38 (m)	120.8					1.47 (m)	
4′		133.8				5″	1.27 (m)	31.7
5′	1.92 (m)	30.5				6″	1.27 (m)	22.5
	2.05 (m)					7″	0.86 (t, 6.8)	14.0
6′	1.53 (m)	27.5						
	1.58 (m)							
2-CH_3	1.16 (s)	23.7						
4′-CH_3	1.64 (br s)	23.6						

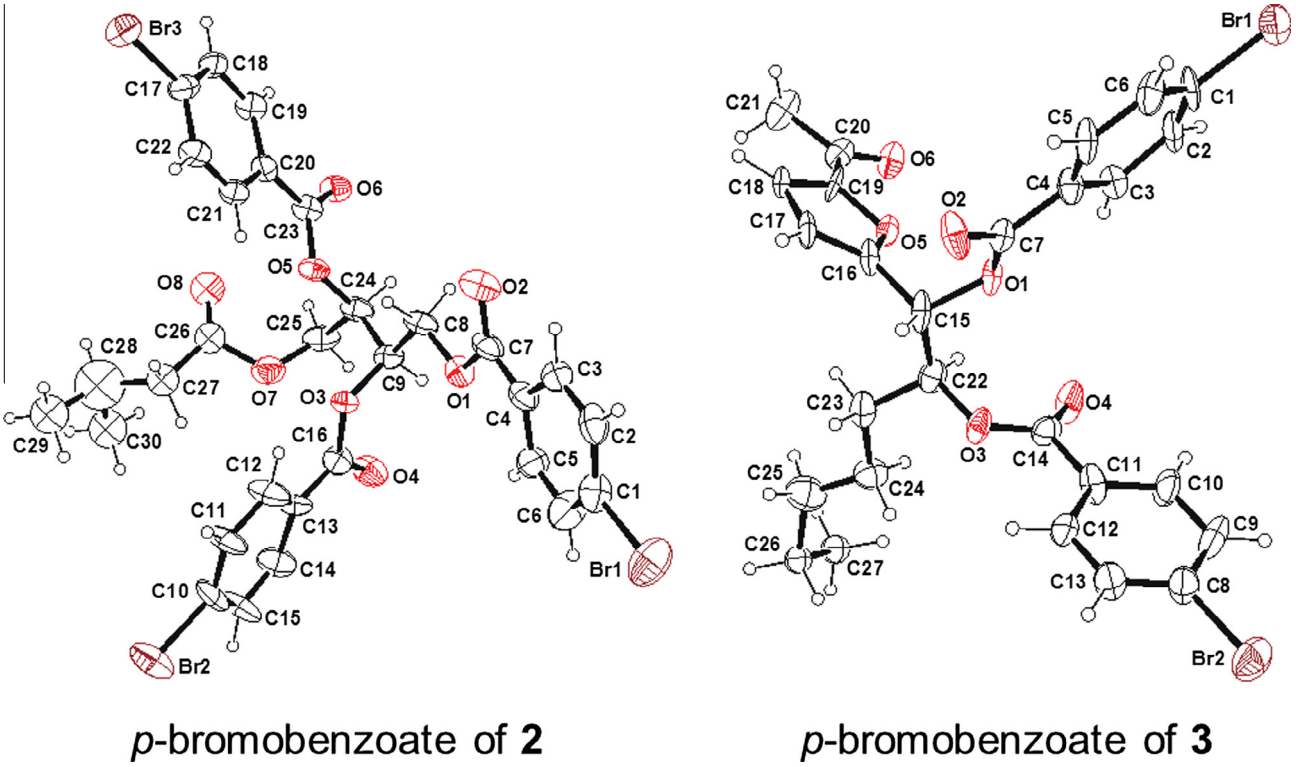

Figure 3. ORTEP drawings of *p*-bromobenzoate of **2** and **3** with ellipsoids at the 30% probability level. Hydrogen atoms are shown spheres of arbitary radii.

analysis of their (*p*-bromo)benzoates (Fig. 3). The Flack parameter ($X = 0.16$ (3)) of 2 in the analysis indicated that its absolute configuration might be as shown in Figure 1. However, the possibility of the opposite configuration could not be excluded. Therefore, the relative configuration of **2** and absolute configuration of **3** were determined to be as shown in Figure 1. The stereochemistry at the asymmetric carbons in **1** remains undetermined.

In order to examine plant growth regulatory activity of these compounds, lettuce was used as the test plant. All the compounds inhibited hypocotyl and root growth of the plant at 1 μmol/paper with significant differences (rate of growth length compared with control ±standard deviation: **1**, root 81.2 ± 11.9%, hypocotyl 55.5 ± 21.9%; **2,** root 68.0 ± 24.6%, hypocotyl 66.3 ± 21.5%; **3**, root 68.3 ± 25.9%, hypocotyl 66.5 ± 21.8%). **1** promoted the hypocotyl growth at 10^{-2} μmol/paper (114 ± 9.15%).

Since Armillaria species mainly infect woody plants and then cause 'Armillaria root disease' to them,[2,3] woody plants should have been used in the bioassay. However, such an assay is very difficult for us because of low quantities of the compounds. Occasionally several herbaceous plants are also damaged by those pathogens.[4] Therefore, the activity of the compounds toward lettuce suggested that armillariols A to C might play some roles in the Armillaria root disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.131.

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