A Novel Fluorescent Dicarboxylic Acid, (*Z*)-1,7-Nonadecadiene-2,3-dicarboxylic Acid, Produced by White-Rot Fungus *Ceriporiopsis subvermispora*

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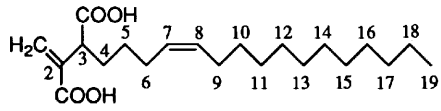
A novel fluorescent dicarboxylic acid bearing a long alkenyl side chain, (*Z*)-1,7-nonadecadiene-2,3-dicarboxylic acid, was isolated from cultures of a white-rot fungus *Ceriporiopsis subvermispora*. This is the first report of a microorganism that produces an amphiphilic itaconic acid derivative with a long aliphatic side chain.

Production of a new aliphatic fungal metabolite, (*Z*)-1,7-nonadecadiene-2,3-dicarboxylic acid (NDA) by a lignin-degrading basidiomycete, *Ceriporiopsis subvermispora* was demonstrated. NDA is an itaconic acid derivative linking a hydrophobic hexadecenyl group at the C-3 position of the core structural unit. Itaconic acid is known to be produced by *Aspergillus itaconicus*,[1] *A. terreus*,[2] *Helicobasidium mompa*,[3] *Ustilago zeae*[4] and *U. maydis*.[5] These fungi produce itaconic acid as a secondary metabolite derived from an intermediate of the tricarboxylic acid (TCA) cycle.[6] In industrial fields, itaconic acid is produced by fermentation by *A. terreus* and used as a monomer of synthetic resins. With regard to the microbial production of dicarboxylic acids having a long aliphatic chain, production of α,ω-dicarboxylic acids, 1,18-octadecenedioic acid by *Candida tropicalis*[7] and α,ω-15,16-dimethyltricotanedioic acid by *Sarcina ventriculi*,[8] were reported. Fomentaric acid, 3-methyl-2,2-dioctadecylbutanedioic acid, is also known as a metabolite of a wood-rotting fungus *Fomes fomentarius*.[9] However there has been no report of itaconic acid derivatives linking a long aliphatic chain. Thus, NDA is classified into a new group of fungal metabolite which has amphiphilic properties due to its hydrophilic itaconate moiety and hydrophobic aliphatic chain.



(Z)-1,7-Nonadecadiene-2,3-dicarboxylic acid

C. subvermispora FP90031 was grown on 5 g of extractive-free beech wood meal containing 15 ml of chelator-free (CF) growth medium in 300 ml Erlenmeyer flasks and incubated at 28 °C. CF medium was prepared by modifying BIII medium.[10] Ammonium sulfate was used as a nitrogen source instead of ammonium tartrate. Nitrilotriacetic acid was omitted from the medium. Glucose (10 g/l) was used as a carbon source. After incubation for 2 weeks, the cultures were washed with Milli-Q^{TM} water and then extracted with a $CHCl_3$/MeOH (2:1) solution. The organic extracts obtained were evaporated to dryness, methylated with diazomethane. The methylated organic extract was purified by silica gel column chromatography and HPLC.[11] The isolated compound was analyzed by

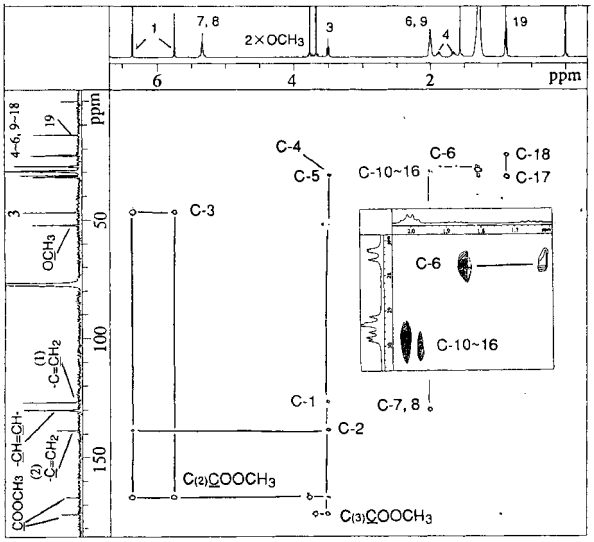


Figure 1. HMBC spectrum of (Z)-1,7-nonadecadiene-2,3-dicarboxylic acid methyl ester.

^{1}H-, ^{1}H-^{1}H COSY, ^{13}C-, HMQC and HMBC NMR, GC-MS and GC.[12]

In ^{1}H-NMR spectrum of NDA (Table 1), two protons of methylene at C-4 give two multiplet signals at δ 1.66 and 1.88 due to a chiral center of C-3. In the HMBC spectrum (Figure 1

Table 1. ^{1}H and ^{13}C NMR data of (Z)-1,7-nonadecadiene-2,3-dicarboxylic acid methyl ester

position	δ^{13}C	δ^1H	HMBC
1	126.8	5.75 s, 6.36 s	H-3
2	138.5		H-1(6.36 ppm), H-3
3	46.7	3.50 t	H-1
4	27.3	1.66 m, 1.88 m	H-3
5	31.3	1.29 m	H-3
6	27.5	2.01 m	H-4
7, 8	129.8, 130.1	5.34 m	H-6, 9
9	27.3	2.01 m	
10~16	29.2~29.9	1.29 m	H-6, 9
17	32.0	1.29 m	H-19
18	22.8	1.29 m	H-19
19	14.2	0.88 t	
2×$COOCH_3$	52.1, 52.2	3.68 s ($C_{(3)}$-$COOCH_3$), 3.77 s ($C_{(2)}$-$COOCH_3$)	
$C_{(2)}$-$COOCH_3$	166.8		H-1, H-3, $C_{(2)}$-$COOCH_3$
$C_{(3)}$-$COOCH_3$	173.9		H-3, $C_{(3)}$-$COOCH_3$

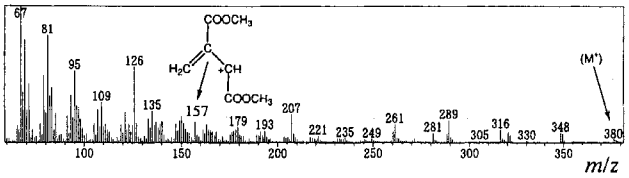


Figure 2. Mass spectrum of (Z)-1,7-nonadecadiene-2,3-dicarboxylic acid methyl ester.

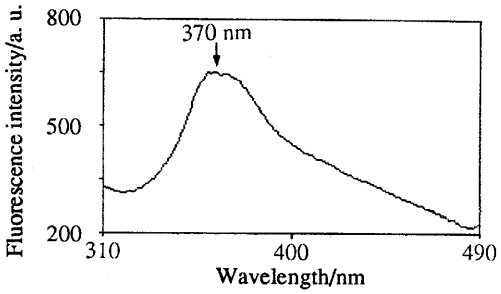


Figure 3. Fluorescence emission spectrum of (Z)-1,7-nonadecadiene-2,3-dicarboxylic acid methyl ester excited at 256 nm.

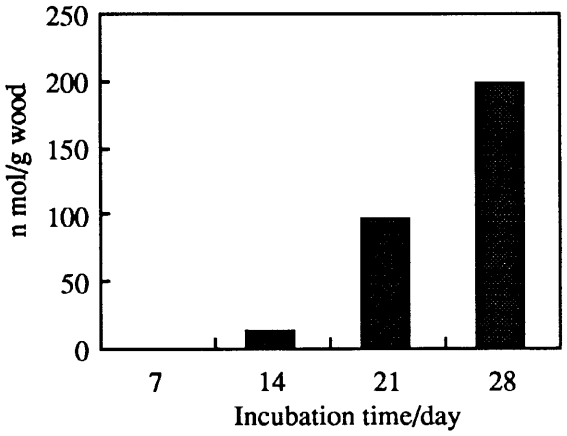


Figure 4. Production of (Z)-1,7-nonadecadiene-2,3-dicarboxylic acid (NDA) by *C. subvermispora* on wood meal/CF cultures.

and Table 1), correlation of H3/C4 was observed at δ 3.50/27.3 and those of H4/C6 at δ 1.66/27.5 and 1.88/27.5. These signal assignments were also ascertained by ^{1}H-^{1}H COSY and HMQC NMR spectra. In GC-MS (Figure 2), a molecular ion and fragment ion of itaconate were observed at *m/z* 380 and 157, respectively.

To determine the configuration of the double bond, coupling constant of olefinic protons (H-7, 8) was measured by spin decoupling of the methylene proton (δ 2.01) vicinal to the double bond. The value of $^{3}J_{\text{H-7,H-8}}$ = 11.5 Hz indicates that this compound is a *cis*-isomer of 1,7-nonadecadiene-2,3-dicarboxylic acid methyl ester. This result is consistent with the chemical shift of C-6 and C-9, δ 27.5 and 27.3, which are typical values for allylic carbon of *cis*-isomer.

The novel fungal metabolite, NDA was found to emit fluorescence, although itaconic acid does not. Fluorescence spectroscopy (RF 1500; Shimadzu) demonstrated that Me-NDA is excited at 256 nm, and emits a pale blue fluorescence at 370 nm (Figure 3). This fluorescence was also observed in organic extract of the cultures of *C. subvermispora*.

Quantitative analysis of NDA by GC demonstrated that the amount of NDA in the wood meal culture of *C. subvermispora* increased gradually (Figure 4).[13] After 4 weeks, it became a major component which was extractable by $CHCl_3$/MeOH. Although NDA was isolated from wood meal cultures, production of NDA in a CF liquid medium without addition of wood meal was also confirmed by GC-MS.

C. subvermispora is known to decompose lignin located in wood cell walls and middle lamellae without penetration of enzymes into wood cell walls.[14,15] To explain lignin-degradation at a site far from the enzymes, lipid peroxidation has been proposed as a possible oxidative process capable of generating potent free radicals.[16-18] Because NDA has a long olefinic chain, it is expected to participate in the extracellular peroxidation. Physiological roles and chemical reactivities of NDA related to lignin degradation are now under investigation.

References and Notes

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10 T. K. Kirk, S. Croan, M. Tien, K. E. Murtagh, and R. L. Farrel, *Enzyme Microb. Technol.*, **8**, 27 (1986).
11 Silica gel column chromatography: hexane/petroleum ether/diethyl ether/acetic acid = 80:13.9:5.9:0.2; HPLC: acetonitrile/methanol/water = 67.5:9.9:22.6, flow rate 0.8 ml/min, Hitachi L-6200 on a Shodex ODP-50 column (250 mm × 6.0 mm i.d., Showa Denko, Japan).
12 NMR: JEOL λ-400 NMR spectrometer (^{1}H : 400 MHz), $CDCl_3$, 22 °C; GC-MS: Shimadzu QP-5050 A on Silicone OV-101 (50 m x 0.25 mm i.d.), GL Science, Japan; GC: Shimadzu GC-14A on the same column.
13 The amount of NDA was calculated by GC using vinyl stearate as an internal standard.
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