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A Novel Maleic Anhydride Derivative from the Fungus *Talaromyces* sp. No. 10092

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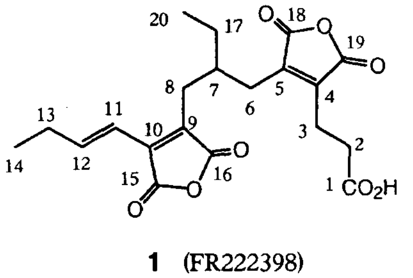
A new maleic anhydride derivative **1** (FR222398) was isolated from fermentations with the fungus *Talaromyces* sp. No. 10092. The structure of **1** was determined based on spectroscopic analysis and chemical conversion. The sodium salt of **1** exhibited a dispersant activity toward kaolin suspension.

Many kinds of naturally occurring biosurfactants have been found from microorganisms, plants and animals.[1—3] Owing to their safe and biodegradable properties, biosurfactants are expected to bring about a new application of surfactants involving that for industrial scale. Spiculisporic acid,[4] obtained from a fungus and developed for an oil-recovery agent, is one of such examples.

During our screening study on biosurfactants from metabolites of the microorganisms, we found that the metabolites of the fungus (*Talaromyces* sp. No. 10092) were shown to be effective in a kaolin suspension.[5] Our continuing efforts have resulted in the isolation of a new maleic anhydride **1**[6] from the metabolites (Chart 1). The pentasodium salt **4**, obtained from **1**, showed a dispersant activity toward a kaolin suspension. This paper describes the isolation and structure determination of **1** based on spectroscopic analysis and chemical conversion.

The fermentation broth[5] was filtered to give a filtrate to which AcOH was added until pH 3, affording precipitates as shown in Fig. 1. The pH of the aqueous solution of the precipitates was adjusted to 7 by the addition of aq NaOH. The solution was then concentrated to dryness to give residue 1, which exhibited a dispersant activity on kaolin. The pH of the aqueous solution of residue 1 was again adjusted to 3 with AcOH to give a suspension, which was extracted with CH_2Cl_2. The organic layer was concentrated to give residue 2, which was suspended in H_2O, and the mixture was neutralized with aq NaOH. This solution showed dispersant activity. Residue 2 was finally purified by HPLC to give compound **1** (colorless powder, $[\alpha]_D+9.0°$), designated as FR222398. The dispersant activity on kaolin was shown by **4** (pentasodium salt of **1**), prepared by the addition of aq NaOH to an aqueous suspension of **1**.

The molecular formula of $C_{20}H_{22}O_8$ for **1** was determined by HREIMS (Found: *m*/*z* 372.1190. Calcd for $C_{20}H_{20}O_7$: ($M^+ - H_2O$) 372.1209) and the ^{13}C NMR spectrum. All 20 carbons appeared in the ^{13}C NMR spectrum,



1 (FR222398)

Chart 1.

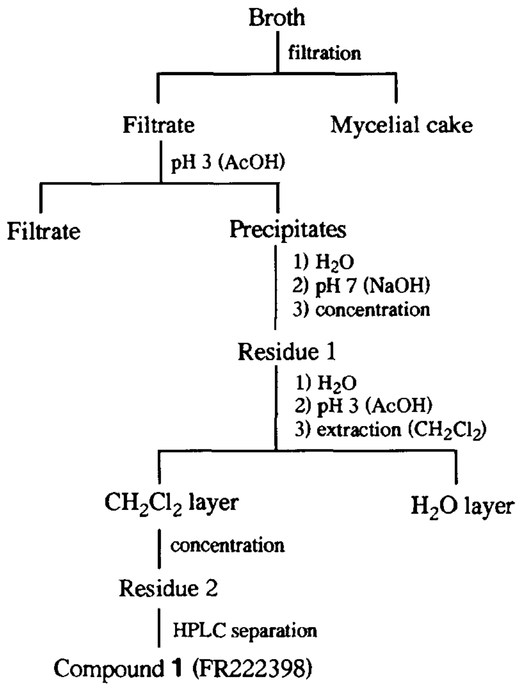


Fig. 1. Isolation procedure.

and DEPT indicated the presence of two methyls, six methylenes, three methines, and nine quaternary carbons (Table 1). The IR spectrum showed absorptions due to carboxylic acid (3500—2600, 1705 cm^{-1}) and acid anhydride (1815, 1770 cm^{-1}). The presence of a cross-conjugated system in **1** was demonstrated by UV absorptions at 255 and 312 nm. The ^1H NMR spectrum of **1** (Table 1) showed two olefinic protons due to a *trans* carbon–carbon double bond at δ = 6.23 (1H, td, J = 1.5, 15.8 Hz) and 7.28 (1H, td, J = 6.6, 15.8 Hz), two methyl groups at δ = 0.98 (3H, t, J = 7.0 Hz) and 1.12 (3H, t, J = 7.4 Hz). The presence of a carboxylic acid group in **1** was confirmed by the formation of methyl ester **2**, which was obtained by the methylation of **1** with diazomethane in ether (Chart 2).

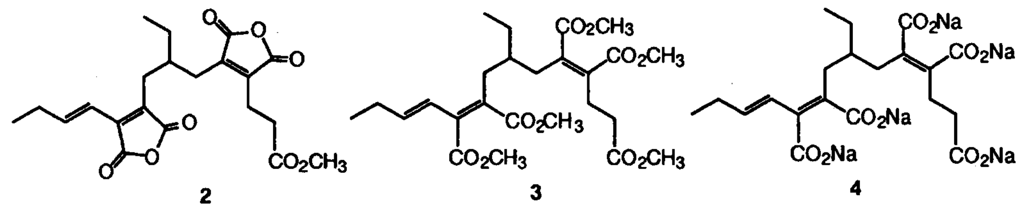
The structure of **1** was deduced by using its pentamethyl ester derivative **3**, which was prepared by the treatment of **1** with excess diazomethane in ether and MeOH (2 : 1). The ^{13}C NMR spectrum of **3** showed 25 carbon signals (Table 1) : seven methyls, six methylenes, three methines, and nine quaternary carbons. The IR spectrum of **3** showed absorptions due to ester groups (1716, 1280 cm^{-1}). The ^1H NMR spectrum showed two *trans* olefinic protons at δ = 6.01 (1H, td, J = 6.6, 15.8 Hz) and 6.31 (1H, td, J = 1.3, 15.8 Hz), five methoxy protons at δ = 3.68 (3H, s), 3.71 (3H, s), 3.72 (3H, s), 3.75 (3H, s) and 3.83 (3H, s), and two methyl groups at δ = 0.91 (3H, t, J = 7.4 Hz) and 1.05 (3H, t, J = 7.4 Hz). ^1H–^1H shift correlation spectroscopy (COSY) demonstrated sequential ^1H-^1H correlations, leading to the three partial structures (A, B, and C), as shown in Fig. 2. After making correlations between ^1H and ^{13}C based on the heteronuclear multiple-quantum coherence (HMQC) (Table 1), these partial structures were connected by heteronuclear multiple bond correlations (HMBC), indicated by the arrows in Fig. 3, providing the gross structure of **3**. The stereochemistry of three carbon–carbon double bonds (4*Z*, 9*Z*, 11*E*) was determined by NOE correlations between H-3 (δ = 2.69) and H-6 (δ = 2.30 and 2.35—2.48), H-8 (δ = 2.39) and H-11 (δ = 6.31), and H-11 and H-13 (δ = 2.24), respectively.

The thus-determined structure of pentamethyl ester **3** strongly suggested the structure of **1** to be the corresponding bis-acid anhydride of **3**. This was confirmed by the chem-

Table 1. NMR Data ($CDCl_3$, δ ppm) for **1** and **3**

Position	^{13}C (125 MHz) for **1**	^1H (500 MHz) for $\mathbf{1}^{a)}$	^{13}C (100 MHz) for **3**	^1H (400 MHz) for $\mathbf{3}^{a)}$
1	177.2 (C)		172.5 (C)	
2	20.0 (CH_2)	2.78 (2H, m)	32.0 (CH_2)	2.44 (2H, t, J = 8.2 Hz)
3	30.9 (CH_2)	2.78 (2H, m)	24.9 (CH_2)	2.69 (2H, t, J = 8.2 Hz)
4	143.6 (C)		136.9 (C)	
5	144.5 (C)		138.1 (C)	
6	28.9 (CH_2)	2.43 (1H, m) 2.58 (1H, m)	33.4 (CH_2)	2.30 (1H, dd, J = 6.9, 13.9 Hz) 2.35—2.48 (1H, m)
7	38.4 (CH)	2.21 (1H, septet, J = 6.9 Hz)	39.5 (CH)	1.63 (1H, septet, J = 6.9 Hz)
8	28.4 (CH_2)	2.38 (1H, m), 2.56 (1H, m)	31.2 (CH_2)	2.39 (2H, m)
9	136.9 (C)		127.3 (C)	
10	138.7 (C)		142.8 (C)	
11	116.1 (CH)	6.23 (1H, td, J = 1.5, 15.8 Hz)	122.6 (CH)	6.31 (1H, td, J = 1.3, 15.8 Hz)
12	150.8 (CH)	7.28 (1H, td, J = 6.6, 15.8 Hz)	143.4 (CH)	6.01 (1H, td, J = 6.6, 15.8 Hz)
13	27.7 (CH_2)	2.33 (2H, m)	26.6 (CH_2)	2.24 (2H, ddq, J = 1.3, 6.6, 7.4 Hz)
14	12.6 (CH_3)	1.12 (3H, t, J = 7.4 Hz)	12.8 (CH_3)	1.05 (3H, t, J = 7.4 Hz)
15	164.3 (C)		169.3 (C)	
16	166.4 (C)		167.5 (C)	
17	26.9 (CH_2)	1.34 (2H, m)	25.9 (CH_2)	1.25—1.35 (2H, m)
18	165.7 (C)		168.7 (C)	
19	165.3 (C)		168.5 (C)	
20	10.9 (CH_3)	0.98 (3H, t, J = 7.0 Hz)	11.0 (CH_3)	0.91 (3H, t, J = 7.4 Hz)
OCH_3			51.8 (CH_3), 52.1 (CH_3) 52.1 (CH_3), 52.2 (CH_3) 52.3 (CH_3)	3.68 (3H, s), 3.71 (3H, s) 3.72 (3H, s), 3.75 (3H, s) 3.83 (3H, s)

a) J in Hz.



2 **3** **4**

Chart 2.

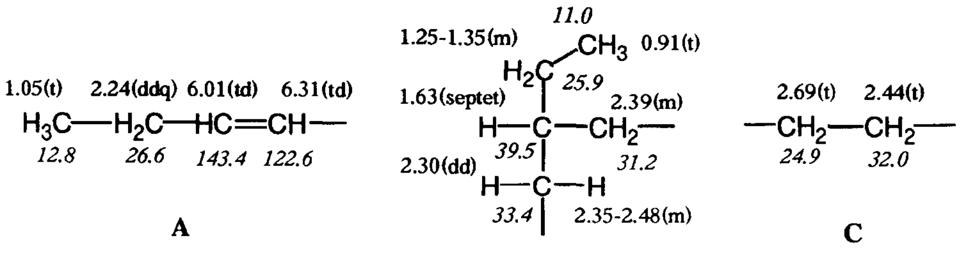


Fig. 2. Partial structures and NMR data for **3**.

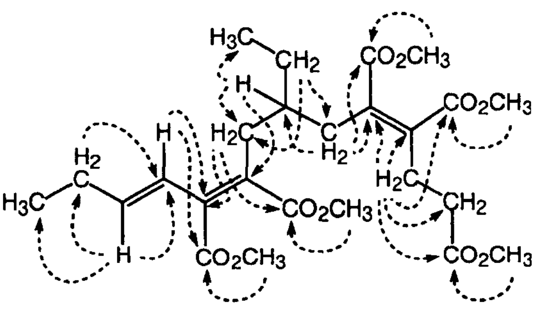


Fig. 3. HMBC correlations in **3**.

ical conversion of **3** to the natural anhydride **1** : treatment of **3** with aq LiOH in THF gave **1**. Thus, the structure of the above-mentioned pentasodium salt was displayed as **4**. The absolute configuration of the chiral center at C-7 has remained unknown.

The interfacial activity of **4** is clearly indicated in Fig. 4. The dispersant activity of **4** toward kaolin slurry is shown in Fig. 5. The activity of **4** was stronger than sodium lignosulfonate, a usual commercial dispersant, and also stronger than sodium gluconate, a salt of carboxylic acid, although the calcium(II) ion chelating activity of **4** was a half degree of that of EDTA 4Na under an alkaline condition (pH 13).

Compound **5**, related structurally to compound **1**, has been found from the present fungus. Compound **5** was also iso-

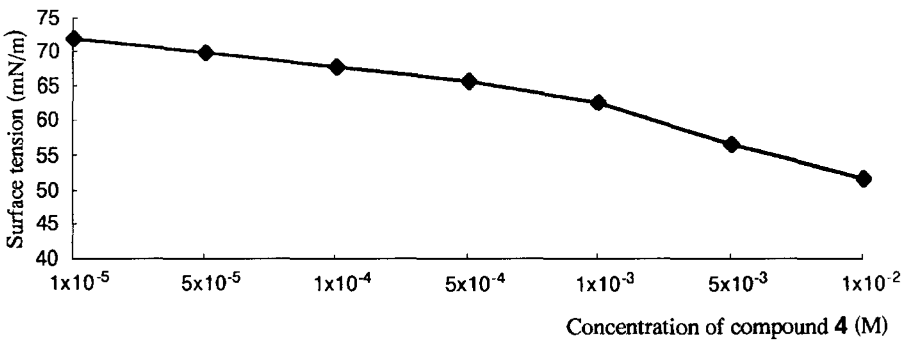


Fig. 4. Effect of **4** on surface tension lowering.

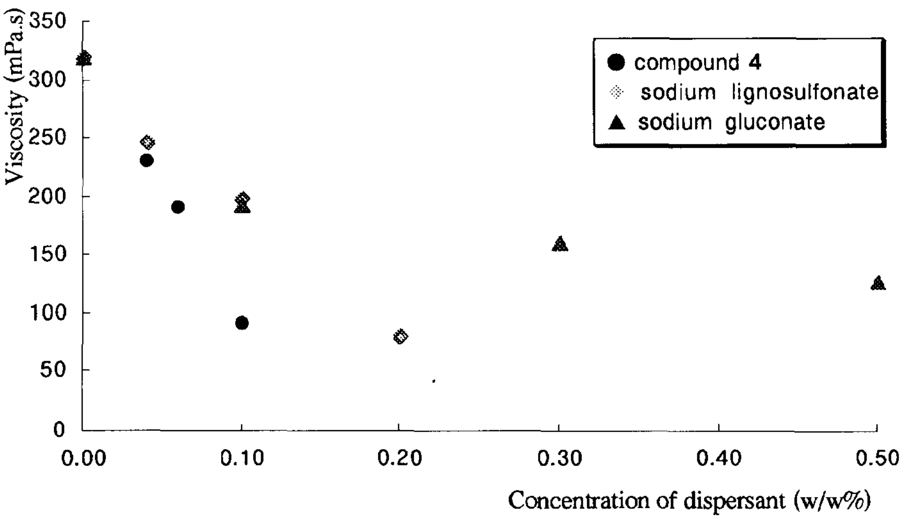


Fig. 5. Dispersant effect of **4** on 40% (w/w) kaolin slurry.

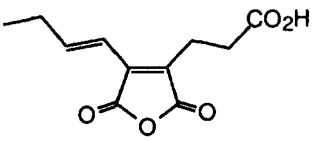


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Chart 3.

lated from the mould, *Paecilomyces varotii* (Chart 3).[7,8]

The new biosurfactant **4**, which has an ability to reduce the viscosity in a kaolin slurry, is expected for applications in dispersion technology in painting, inorganic materials and synthetic resins as a safe and biodegradable dispersant.

Experimental

General method. Optical rotations were measured in a $CHCl_3$ solution at 25 °C with a HORIBA SEPA-300 automatic polarimeter. IR spectra were recorded with a Shimadzu IR-400 spectrophotometer and UV spectra with a Hitachi 150-20 spectrophotometer. NMR spectra were recorded with a Bruker DRX-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) and a Bruker DRX-400 (^{1}H, 400 MHz ; ^{13}C, 100 MHz) spectrometer. Two-dimensional (2D) NMR spectra, COSY, ^{1}H-^{13}C COSY, HMQC, HMBC and NOESY were measured using standard Bruker pulse sequences. The chemical shifts are expressed on a δ ppm scale with $CHCl_3$ (^{1}H, 7.26 ppm) and $CDCl_3$ (^{13}C, 77.0 ppm) in $CDCl_3$, and with 3-(trimethylsilyl)propionic-2, 2,3,3-d_4 acid sodium salt (TSP) in D_2O as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). The multiplicities of the ^{13}C resonance were achieved by DEPT experiments, which were performed using polarization transfer pulses of 90° and 135°, first obtaining only signals for CH groups and then positive signals for CH and CH_3 and negative signals for CH_2 groups. Mass spectra (MS) and high-resolution electron impact MS (HREIMS) were taken with a Micromass Auto Spec spectrometer.

Column chromatography was carried out on Merck Silica gel 60 (70—230 mesh) and preparative thin-layer chromatography was performed on Merck Silica gel 60 F_{254} plates. Medium-pressure liquid chromatography (MPLC) was carried out with a KHLC-201-43 (Kusano) apparatus using a CIG prepacked column (ODS silica gel, CPO-HS-221-20, for reversed phase). HPLC was conducted with a YMC-Pack ODS-A (ODS, 525-15-SR) for reversed phase.

Isolation and purification. To the filtrate (2 L) of the fermentation broth[5] was added AcOH (7 L) until pH 3, providing precipitates. The filtered precipitates were dissolved in H_2O and the solution was neutralized to pH 7 by adding a 0.1 M (1 M = 1 mol dm^{-3}) aqueous NaOH solution. The solution was concentrated to dryness to obtain residue 1 (20.2 g), which was dissolved in H_2O. After being adjusted to pH 3 with AcOH, the mixture was extracted with CH_2Cl_2 three times (2×1 L and then 0.5 L). The combined CH_2Cl_2 soluble portions were concentrated under reduced pressure to give residue 2 as a yellow oil (10.2 g), which was subjected to reversed-phase HPLC using a CH_3CN–phosphate buffer solution (6 : 4) as an eluant; a phosphate buffer solution was prepared by solving KH_2PO_4 (6.53 g) and H_3PO_4 (1.18 g) in H_2O (3 L). After the evaporation of CH_3CN of the pure fractions, the resulting suspension was extracted with AcOEt. The AcOEt soluble portion was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure to give compound **1** (FR222398) (2.0 g).

Compound 1 (FR222398). Colorless powder. $[\alpha]_D$ +9.0° (c 0.5, $CHCl_3$). EIMS m/z 372 (M^+—H_2O). HREIMS Found: m/z 372.1190. (M^+—H_2O, Calcd for $C_{20}H_{20}O_7$: (M^+-H_2O), 372.1209). Found: C, 61.76; H, 5.81%. Calcd for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68%. IR (film) ν_{max} 3500—2600, 1815, 1770, 1705 cm^{-1}. UV (CH_3CN) λ_{max} 255 (ε 8300), 312 (ε 11000) nm. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) are listed in Table 1.

Preparation of 2. Excess diazomethane in ether was added to a solution of **1** (20 mg) in ether (1 mL) at 0° C, and the mixture was stirred at room temperature for 1h. The mixture was concentrated under reduced pressure. The obtained residue was purified by silica-gel column chromatography (hexane–AcOEt = 4 : 1 as an eluant) to give **2** (12 mg).

Compound 2. Yellow viscous oil. IR (film) ν_{max} 1822, 1766, 1650, 1275 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.98 (3H, t, J = 7.4 Hz), 1.12 (3H, t, J = 7.4 Hz), 1.35 (2H, qd, J = 7.0, 7.4 Hz), 2.21 (1H, br septet, J = 7.0 Hz), 2.33 (2H, ddq, J = 1.6, 6.4, 7.4 Hz), 2.38 (1H, dd, J = 7.0, 14.0 Hz), 2.47 (1H, dd, J = 6.7, 13.8 Hz), 2.57 (1H, dd, J = 7.4, 13.8 Hz), 2.59 (1H, dd, J = 7.8, 14.0 Hz), 2.65—2.79 (4H, m), 3.66 (3H, s), 6.22 (1H, td, J = 1.6, 15.8 Hz), 7.28 (1H, td, J = 6.7, 15.8 Hz).

Preparation of 3. Excess diazomethane in ether was added to a solution of **1** (80 mg) in a mixed solvent of ether (2 mL) and MeOH (1 mL) at 0 °C. The mixture was stirred at room temperature for 16 h, and concentrated under reduced pressure. The obtained residue was purified by preparative TLC to give **3** (21 mg).

Compound 3. Pale-yellow viscous oil. $[\alpha]_D$ −7.7° (c 0.7, $CHCl_3$). EIMS m/z 496 (M^+). HREIMS Found: m/z 465.2119. Calcd for $C_{24}H_{33}O_9$: (M^+-CH_3O), 465.2124. IR (film) ν_{max} 1716, 1433, 1280 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) are listed in Table 1.

Hydrolysis of 3. To a solution of **3** (5.5 mg) in THF (0.5 mL) was added 1M LiOH aqueous solution (0.5 mL). The mixture was allowed to stand at room temperature for 1.5 h. To the mixture, CH_2Cl_2 (10 mL), AcOH (1 mL) and H_2O (5 mL) were successively added, and the mixture was stirred for several minutes. After the CH_2Cl_2 layer was separated, the residual aqueous layer was extracted twice with CH_2Cl_2 (10 mL). The combined CH_2Cl_2 soluble portions were concentrated under reduced pressure, and the obtained residue was subjected to reversed-phase MPLC (CH_3CN–H_2O = 1 : 1, then CH_3CN) to give **1** (2.4 mg).

Preparation of 4. An aqueous suspension of **1** (206 mg) in H_2O (100 mL) was neutralized to pH 7 by adding a 1 M NaOH aqueous solution. The mixture was concentrated to dryness under reduced pressure to give pentasodium salt **4** (304 mg).

Compound 4. Pale-yellow viscous oil. IR (film) ν_{max} 3700—2600, 1660, 1630, 1560, 1400 cm^{-1}. ^{1}H NMR (400 MHz, D_2O) δ = 0.86 (3H, t, J = 7.3 Hz), 1.00 (3H, t, J = 7.4 Hz), 1.33 (2H, m), 1.54 (1H, m), 2.15—2.45 (8H, m), 2.49—2.57 (2H, m), 5.87 (1H, td, J = 6.6, 15.8 Hz), 6.38 (1H, d, J = 15.8 Hz). ^{13}C NMR (100 MHz, D_2O) δ = 12.9 (CH_3), 15.7 (CH_3), 27.4 (CH_2), 28.8 (CH_2), 29.7 (CH_2), 35.3 (CH_2), 36.4 (CH_2), 39.1 (CH_2), 41.3 (CH), 126.6 (CH), 134.9 (C), 139.8 (C), 140.4 (C), 141.2 (CH), 143.3 (C), 180.6 (C), 181.6 (C), 181.7 (C), 181.8 (C), 185.4 (C).

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