Journal of Natural Products
Vol. 58, No. 3, pp. 463-466, March 1995

BIPOLAL: ANTI-MICROALGAL COMPOUND ISOLATED AS A CANDIDATE FOR MARINE ANTIFOULING PRODUCED BY *BIPOLARIS* SP. F5206

NAOHARU WATANABE,* AKIKO FUJITA, NOBUYOSHI BAN, AKIHITO YAGI, HIDEO ETOH, KAZUO INA,

Department of Applied Biological Chemistry, Shizuoka University, 836 Ohya, Shizuoka 422, Japan

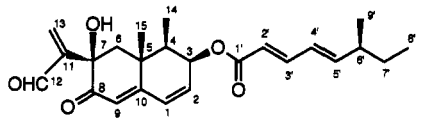
and KANZO SAKATA

Research Laboratory of Marine Biological Science, Faculty of Agriculture, Shizuoka University, Mochimune, Shizuoka 422, Japan

ABSTRACT.—Bipolal [**1**], a new compound with marine antifouling potential, was isolated from the culture broth of *Bipolaris* sp. The structure was determined to be an eremophilane derivative on the basis of 2D nmr spectroscopy and chemical transformations.

In the course of our screening program for marine antifouling compounds, we have reported that ideal antifouling substances inhibit the initial attachment and growth of all attaching organisms such as microorganisms, diatoms, and larvae of sessile animals, prevent microfilm formation, and maintain the antifouling effect for more than three months. We designed a laboratory-matching screening system using diatoms, marine bacteria, and a fungus, as a time-saving assay for marine antifouling compounds (1,2). Our efforts to discover new antifouling substances by screening culture broths of microorganisms have led to the isolation of the new compound bipolal [**1**] as a candidate for marine antifouling. This paper deals with the production, isolation, and structure elucidation of **1**.

The whole broth (1 liter) of a fungus identified as *Bipolaris* sp. (Hyphomycetes) was extracted with EtOAc. Isolation was guided by antimicroalgal activity and inhibitory activity against a marine Gram-positive bacterial strain. The organic extract was evaporated and the concentrate was chromatographed on Si gel (hexane/EtOAc), Sephadex LH-20 (MeOH), and Si gel (C_6H_6/Me_2CO) columns to give the white crystalline solid bipolal [**1**] as an antidiatom compound. The molecular formula of **1** was determined as $C_{24}H_{30}O_5$ by hrfabms in conjunction with its ^{1}H- and ^{13}C-nmr spectral data (Table 1). All of the ^{1}H- and ^{13}C-nmr data of **1** were unambiguously assigned by ^{13}C-^{1}H COSY and ^{1}H-^{1}H COSY experiments and are shown in Table 1. The DEPT and ^{13}C-^{1}H COSY nmr spectra of **1** indicated 24 carbons and 29 carbon-bound hydrogens. The carbon types included 4×CH_3, 2×CH_2, 2×CH, 1×O-CH, 1×>C<, 1×O-C<, 10 olefinic carbons (one of them is an exomethylene carbon), two carbonyls, and one formyl group.



1

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$, Table 1) of **1** showed a formyl signal at δ 9.53 for H-12, two exomethylene protons at δ 6.32 (s) and 6.85 (s) for H_2-13, as well as four *E*-coupled conjugated olefinic protons (H-2′, 3′, 4′, and 5′), two *Z*-olefinic protons (H-1 and H-2), and one singlet olefinic proton (H-9), together with one OH and sp^3-carbons bearing 19 protons. The ^{1}H-nmr analysis and ^{1}H-^{1}H COSY spectrum of **1** revealed the sequential connectivities (Figure 1, solid lines) of C-2′-3′-4′-5′-6′-(9′)-7′-8′ (6-methylocta-2,4-dienoic acid moiety) and C-1-2-3-4-14. The

TABLE 1. ^{13}C- (75 MHz) and ^{1}H-Nmr (300 MHz) Data for Bipolal [**1**] in $CDCl_3$.

Position	^{13}C (mult.)	^{1}H (mult., J in Hz)	Position	^{13}C (mult.)	^{1}H (mult., J in Hz)
1	131.1 (d)	6.42 (d, 9.8)	14	10.5 (q)	1.01 (3H, d, 7.2)
2	133.1 (d)	6.29 (dd, 9.8, 5.2)	15	23.0 (q)	1.51 (s)
3	68.9 (d)	5.44 (dd, 5.2, 5.2)	1′	166.8 (s)	
4	40.9 (d)	1.99 (m)	2′	118.8 (d)	5.81 (d, 15.3)
5	36.2 (s)		3′	146.0 (d)	7.25 (dd, 15.3, 10.6)
6	44.5 (t)	Ha 2.00 (m), Hb 2.09 (d, 14.3)	4′	126.7 (d)	6.16 (dd, 10.6, 16.2)
7	74.8 (s)		5′	150.9 (d)	6.03 (dd, 16.2, 7.6)
8	197.1 (s)		6′	38.8 (d)	2.18 (m)
9	124.0 (d)	6.01 (s)	7′	29.3 (t)	1.38 (2H, dq, 7.6, 7.3)
10	161.9 (s)		8′	12.0 (q)	0.87 (3H, t, 7.3)
11	154.6 (s)		9′	19.5 (q)	1.03 (3H, d, 6.7)
12	192.7 (d)	9.53 (s)	OH-7		2.52
13	135.8 (t)	Ha 6.32 (s), Hb 6.85 (s)			

COLOC nmr spectrum showed cross-peaks (Figure 1, arrows) at: H-2 to C-10; H-3 to C-1, -2, -5; H-4 to C-6; H-6b to C-8; H-9 to C-1, -7, -8; H_3-14 to C-3, -4, -5; and H_3-15 to C-4, -5, -6, leading to a 1,2,6,7,8,8a-hexahydro-1,8a-dimethyl-6-oxo-naphthalene (eremophilane) skeleton. Long-range couplings from H-2′, H-3′, and H-3 to C-1′ revealed the ester linkage of the 6-methylocta-2,4-dienoic acid group to the eremophilane group at C-3. Cross-peaks from one of the exomethylene protons δ 6.85 (H-13a) to C-11 and C-12 indicated the presence of a 1-formyl ethenyl group, which connects at the deshielded quaternary carbon (C-7, δ 74.8) based on the long-range couplings from the formyl proton and one of the exomethylene protons to C-7. The cross-peak from OH to C-6 indicated that the OH group is located at C-7. Thus, the planar structure of **1** was determined.

NOes (Figure 1, dotted curves) between two sets of protons (7-OH, H_3-14, H_3-15, and H-3, H-4) indicated that these sets of protons are on the same face of the eremophilane skeleton. Although the α,β-unsaturated carbonyl due to the formyl ethenyl group was located close to one of the α,β,γ,δ-unsaturated carbonyl systems, the cd spectrum of **1** showed a positive maximum (Δε +44) at 282 nm and a negative one (Δε −19) at 255 nm, indicating clockwise rotation of the two conjugated systems at the chiral center C-3 (3,4). To confirm the absolute stereochemistry, **1** was treated with alkaline MeOH to afford trinoreremophilane [**2**] and methyl 6-methylocta-2,4-dienoate [**3**] (Scheme 1). The same exciton splitting pattern in the cd spectrum of **2** (Δε +57 at 283 nm, Δε −21 at 254 nm) as that of **1** confirmed the C-3 configuration of **1** and **2** to be *S*.

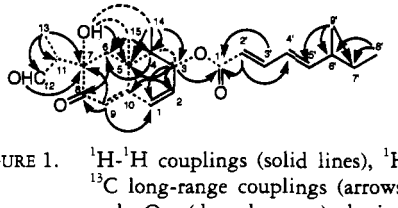
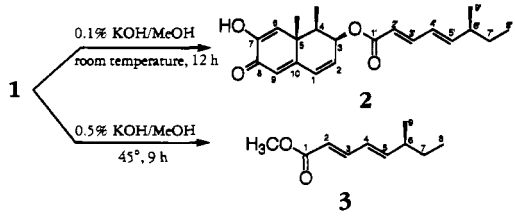


FIGURE 1. ^{1}H-^{1}H couplings (solid lines), ^{1}H-^{13}C long-range couplings (arrows), and nOes (dotted curves) obtained from the ^{1}H-^{1}H COSY, COLOC, and differential nOe nmr spectra of bipolal [**1**].

The chirality of C-6′ was determined to be *S* based on an $[\alpha]_D$ of +28° of **3** by comparison with that of methyl (6*S*,2*E*,4*E*)-6-methylocta-2,4-dienoate (5). Thus, the structure of bipolal was determined as (1*R*,2*S*,7*R*,8a*R*)-1,2,6,7,8,8a-hexahydro-7-[1-(formyl)ethenyl]-7-hydroxy-1,8a-dimethyl-6-oxonaphthalen-2-yl (6*S*,2*E*,4*E*)-6-methylocta-2,4-dienoate [**1**].

Although the structurally similar dendryphiellins have been isolated from the culture broth of a marine Deuteromycete (*Dendryphiella* sp.) (5–7), bipolal [**1**] is, to the best of our knowledge, a new compound. Bipolal [**1**]



SCHEME 1. Conversion of **1** to **2** and **3** by treatment with KOH/MeOH.

showed MIC values of 6.25 and 1.58 μg/ml against Gram-positive marine bacterial strain No. 38 and *Trichophyton mentagrophytes*, respectively. The growth of the diatom *Nitzschia closterium* was also inhibited to 12% at a concentration of 1.6×10^{-2} μmol/ml. We have already reported antimicroalgal compounds with antimicrobial activity against the marine Gram-positive bacterial species, *Trichophyton mentagrophytes*, that exhibited antifouling activity (1,2). Thus, bipolal [**1**] can be regarded as a candidate marine antifouling substance.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ with TMS as internal standard, employing JEOL JNM-GX400 and Bruker AM-300 spectrometers. The fabms and hrfabms were recorded on a JEOL JMS-DX303 HF spectrometer. A Jasco J-500A spectrometer was used for measuring cd spectra.

BIOASSAYS.—*Trichophyton mentagrophytes* and the marine Gram-positive bacterial strain No. 38, isolated from the biofilm on the submerged assay plate, were used for conventional antimicrobial tests using a micro-broth dilution method (2). The attaching diatom, *Nitzschia closterium* (collected from Hamana Lake, Shizuoka, Japan), was used for the antimicroalgal assay (2).

CULTURE STRAIN AND GROWTH CONDITIONS.—The producing microorganism strain F5206 was isolated from a fallen leaf sample. It has dark brown conidiophores, producing conidia through apical pores and forming dark brown conidia on successive new tips on malt-dextrose agar plates. Based on these observations the strain F5206 has been identified as *Bipolaris* sp. according to the descriptions of Barnett and Hunter (8). One loopful of cells of the strain F5206 from an agar slant was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of medium consisting of (w/v): glucose 2.0%, yeast extract 0.2%, polypepton 0.05%, $MgSO_4.7H_2O$ 0.5%, and KH_2PO_4 0.1%. The pH was adjusted to 6.0 prior to sterilization. Fermentation proceeded for 72 h at 25° on a rotary shaker operating at 200 rpm. A 250-ml quantity of the culture was inoculated in a 5-liter Erlenmeyer flask containing 1 liter of the above-mentioned medium. The fermentation was carried out for 120 h under the same conditions as described above.

PURIFICATION AND ISOLATION.—The whole broth (1 liter) was extracted twice with 500 ml of EtOAc. The organic extracts were dried over Na_2SO_4, filtered, and evaporated *in vacuo*. Bipolal [**1**] was isolated by monitoring its antidiatom activity. The oily residue from the EtOAc extract was subjected to Si gel cc (hexane/EtOAc). The biologically active fraction (hexane-EtOAc, 80:20 to 50:50) was concentrated, and purified by Sephadex LH-20 (MeOH) and Si gel (C_6H_6/Me_2CO) cc to give the antidiatom compound **1** (50 mg).

Bipolal [**1**].—White crystalline solid, mp 108–110°; $[\alpha]^{24}D$ +364° (*c*=1.0, EtOH); uv (EtOH) λ max (ε) 276 (25,000), 210 (11,000) nm; ir (KBr) ν max 3435, 1713, 1693, 1664 cm^{-1}; hrfabms *m/z* 399.2155 $[M+H]^+$, calcd for $C_{24}H_{31}O_5$, 399.2171, Δ −1.6 mmu; ^{1}H-nmr (300 MHz, $CDCl_3$) and ^{13}C-nmr (75 MHz, $CDCl_3$) data, see Table 1.

(1R,2S,8aR)-1,2,6,8a-Tetrahydro-7-hydroxy-1,8a-dimethyl-6-oxonaphthalen-2-yl (6S,2E,4E)-6-methylocta-2,4-dienoate [**2**].—Treatment of **1** (2 mg) with 0.1% KOH in MeOH at room temperature for 12 h afforded **2**: 0.8 mg; fabms *m/z* 343 $[M+H]^+$; ir (neat) ν max 1641, 1710 cm^{-1}; uv (MeOH) λ max 269, 215 nm; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 6.45 (d, *J*=10.0 Hz, H-1), 6.34 (s, OH-7), 6.29 (s, H-6), 6.26 (m, H-3′), 6.20 (s, H-9), 6.19 (dd, *J*=10.0 and 4.8 Hz, H-2), 6.17 (dd, *J*=10.8 and 15.6 Hz, H-4′), 6.04 (dd, *J*=15.6 and 7.6 Hz, H-5′), 5.83 (d, *J*=14.8 Hz, H-2′), 5.55 (dd, *J*=4.8 and 4.8 Hz, H-3), 2.19 (m, H-6′), 2.10 (m, H-4), 1.44 (s, CH_3-5), 1.38 (dq, *J*=7.2 and 7.2 Hz, H_2-7), 1.17 (d, *J*=7.2 Hz, CH_3-4), 1.04 (d, *J*=6.8 Hz, CH_3-9′), 0.87 (t, *J*=7.6 Hz, CH_3-8′).

Methyl (6S,2E,4E)-6-methyloctane-2,4-dienoate [**3**].—Reaction of **1** (10 mg) with 0.5% KOH in MeOH at 45° for 9 h gave methyl 6-methylocta-

2,4-dienoate [**3**]: 2 mg; $[\alpha]^{24}D$ +28° (c=0.2, EtOH); fabms m/z 169 $[M+H]^+$; ^1H nmr ($CDCl_3$, 400 MHz) δ 7.30 (dd, J=15.4 and 10.8 Hz, H-3′), 6.14 (dd, J=15.4 and 10.8 Hz, H-4′), 6.02 (dd, J=15.4 and 7.4 Hz, H-5′), 5.80 (d, J=15.4 Hz, H-2′), 3.74 (s, OCH_3), 2.17 (m, H-6′), 1.37 (dq, J=7.4 and 7.4 Hz, H_2-7′), 1.02 (d, J=7.4 Hz, CH_3-9′), 0.87 (t, J=7.4 Hz, CH_3-8′).

ACKNOWLEDGMENTS

The authors wish to thank the Research Center of Taisho Pharmaceutical Co., Ltd., for providing terrestrial microorganisms for the screening.

LITERATURE CITED

1. R. Takasawa, C. Ogura, N. Watanabe, H. Etoh, K. Sakata, and K. Ina, *Biosci. Biotech. Biochem.*, **56**, 1208 (1992).
2. N. Watanabe, A. Watanabe, C. Ogura, H. Etoh, K. Sakata, K. Okamoto, T. Kimura, and K. Ina, *Biosci. Biotech. Biochem.*, **57**, 1747 (1993).
3. N. Harada, *J. Am. Chem. Soc.*, **95**, 240 (1973).
4. M. Koreeda, N. Harada, and K. Nakanishi, *J. Am. Chem. Soc.*, **96**, 266 (1974).
5. A. Guerriero, M. D'Ambrosio, V. Cuomo, F. Vanzanella, and F. Pietra, *Helv. Chim. Acta*, **72**, 438 (1989).
6. A. Guerriero, M. D'Ambrosio, V. Cuomo, F. Vanzanella, and F. Pietra, *Helv. Chim. Acta*, **71**, 57 (1988).
7. A. Guerriero, V. Cuomo, F. Vanzanella, and F. Pietra, *Helv. Chim. Acta*, **73**, 2090 (1990).
8. H.L. Barnett and B.B. Hunter, "Illustrated Genera of Imperfect Fungi," MacMillan Publishing Company, New York, 1987, p. 126.

Received 6 September 1994