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NEW ANTIFUNGAL BITHIENYLACETYLENES FROM *BLUMEA OBLIQUA*

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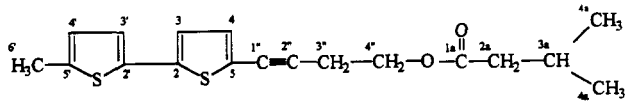
ABSTRACT.—Two new bioactive bithienylacetylenes isolated from *Blumea obliqua* have been characterized as 5′-methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene [**1**] and 5′-hydroxymethyl-5-[butyl-3-en-1-yn]-2,2′-bithiophene isovaleroxy ester [**2**], respectively. Compounds **1** and **2** showed antifungal activity against *Epidermophyton floccosum* and *Pleurotus ostreatus*.

Blumea obliqua (L.) Druce (syn. *Blumea amplectens* DC.) belongs to the Asteraceae (tribe Inuleae) and grows as an annual herb in coastal areas of Pakistan and India (1,2). *B. obliqua* is used for the treatment of various ailments in folk medicine (3). Plants of this genus are also used as remedies for malaria, influenza, bronchitis, and asthma (4). Earlier studies of this species have resulted in the isolation of a thiophene acetylene derivative, amplectol, and a few other known compounds (5,6).

Many naturally occurring and synthetic acetylenic thiophenes have been found to possess uv-mediated antimicrobial activity. Acetylenic thiophenes have shown very good antimicrobial activity against *Candida albicans* and *Staphylococcus albus* only when irradiated with uv-A light (7,8). Thiophenic compounds having 1-alkynyl groups linked to a thiophene in the α-position, or to a 2,2′-bithienyl system in the 5-position have exhibited the most potent uv-mediated antibiotic activity (9). These compounds were also found to possess photoactive antiviral and cytotoxic activities (10). The compounds possessing a conjugated linear configuration of two or three thiophene rings and a substituent with an acetylenic linkage were found to be the most active. A naturally occurring terthiophene, α-terthiophene, has shown anti-HIV activity on irradiation with uv-A light (11).

In the continuation of our search for biologically active compounds from *B. obliqua* (6), we selected a petroleum ether-soluble part of the plant extract which exhibited strong antifungal activity against various fungi. Cc on Si gel, followed by prep. tlc of the petroleum ether extract, yielded two new bithienylacetylenes, 5′-methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene [**1**] and 5′-hydroxymethyl-5-[butyl-3-en-1-yn]-2,2′-bithiophene isovaleroxy ester [**2**]. Their structures were elucidated on the basis of extensive spectroscopic studies including 1D, 2D, and inverse 2D nmr spectroscopic techniques.

5′-Methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene [**1**] was isolated as a yellow oil. The hreims of **1** showed the molecular ion peak at *m/z*



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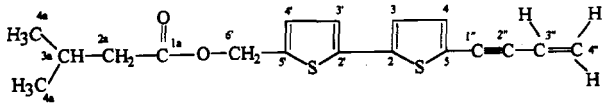

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332.0903, which corresponded to the molecular formula $C_{18}H_{20}O_2S_2$ and indicated the presence of nine double-bond equivalents. A strong $[M+2]^+$ peak in the eims indicated the presence of sulfur. The uv spectrum showed an absorption maximum at 339 nm (ϵ=26,720) indicating the presence of a bithiophene-acetylene system (12,13). The ir spectrum exhibited a strong absorption band at 1717 (ester carbonyl) cm^{-1}. The ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz) of **1** showed a six-proton doublet at δ 0.97 (J=6.5 Hz), a one-proton multiplet at δ 2.12, and a two-proton doublet at δ 2.22 (J=6.5 Hz). These values, along with the loss of 102 mass units (2-methylbutanoic acid) from the molecular ion and a strong band for an ester carbonyl in the ir spectrum revealed the presence of an isovaleroxy functionality. The signals at δ 2.78 (2H, t, J=6.7 Hz) and 4.24 (2H, t, J=6.7 Hz) were assigned to the C-3″ and C-4″ protons, respectively. Thiophenic protons and the C-6′ protons were assigned on the basis of a COSY-45° nmr spectrum and the various proton-carbon ^{1}H/^{13}C connectivities were established with a HMQC experiment (Table 1).

The ^{13}C-nmr spectrum of **1** showed the presence of 18 carbon atoms. The DEPT spectrum indicated the presence of five methines, three methylenes, and three methyl carbons, and the presence of seven quaternary carbons was deduced in the molecule. Their ^{13}C-nmr chemical shifts (Table 1) (14) were assigned on the basis of the HMBC spectrum.

5′-Hydroxymethyl-5-[butyl-3-en-1-yn]-2,2′-bithiophene isovaleroxy ester [**2**] was isolated as a light yellow oil. The hreims showed a molecular ion peak at *m/z* 330.0750, which was in agreement with a molecular formula of $C_{18}H_{18}O_2S_2$ and indicated the presence of ten degrees of unsaturation in the molecule. Again, a strong $[M+2]^+$ peak indicated the presence of sulfur in the compound. The uv

TABLE 1. ^{13}C-Nmr Data and ^{1}H/^{13}C Connectivities for Compounds **1** and **2** in $CDCl_3$.

Position	Compound					
	1			**2**		
	Chemical Shift (δ)	Multiplicity (DEPT)	^{1}H/^{13}C Connectivity (HMQC) (J Hz)	Chemical Shift (δ)	Multiplicity (DEPT)	^{1}H/^{13}C Connectivity (HMQC) (J Hz)
2	138.6	C	—	138.6	C	—
3	122.5	CH	6.88 (d, J=3.7)	123.7	CH	7.01[a] (d, J=3.5)
4	132.5	CH	6.99 (d, J=3.7)	132.8	CH	7.07 (d, J=3.5)
5	121.6	C	—	122.2	C	—
2′	134.5	C	—	137.9	C	—
3′	124.3	CH	6.93 (d, J=3.5)	123.7	CH	7.00[a] (d, J=3.5)
4′	126.0	CH	6.64 (dq, J=3.5, 1.0)	128.8	CH	6.96 (d, J=3.5)
5′	139.8	C	—	138.0	C	—
6′	15.3	Me	2.48 (d, J=1.0)	60.2	CH_2	5.2 (s)
1″	75.3	C	—	83.1	C	—
2″	90.5	C	—	93.1	C	—
3″	20.4	CH_2	2.78 (t, J=6.7)	116.8	CH	6.01 (dd, J=17.5 11.0)
4″	60.6	CH_2	4.24 (t, J=6.7)	127.0	CH_2	5.54 (dd, J=11.0, 1.5)
4″	—	—	—	127.0	CH_2	5.72 (dd, J=17.5, 1.5)
1a	172.8	C	—	172.2	C	—
2a	43.4	CH_2	2.22 (d, J=6.5)	43.2	CH_2	2.21 (d, J=7.0)
3a	25.8	CH	2.12 (m)	25.7	CH	2.12 (m)
4a	22.4	2×Me	0.97 (d, J=6.5)	22.4	2×Me	0.94 (d, J=7.0)

[a]Assignments are interchangeable.

spectrum of **2** showed an absorption maximum at 344 nm (ϵ=27,620) due to the presence of a conjugated bithiopheneacetylenic chromophore (15). The ir spectrum displayed strong absorption bands at 2840 (CH) and 1725 (ester carbonyl) cm^{-1}.

The ^{1}H-nmr spectrum revealed the presence of an isovaleroxy function, and a loss of 101 mass units from the molecular ion (2-methylbutanoic acid) confirmed this functionality. Three doublets of doublets at δ 6.01 (J=17.5 and 11.0 Hz), 5.72 (J=17.5 and 1.5 Hz), and 5.54 (J=11.0 and 1.5 Hz), indicative of the presence of an unsubstituted terminal methylene, and a singlet at δ 5.20 instead of a doublet at δ 2.48, indicated that the isovaleroxy function was affixed to C-6′ rather than C-4″ as was the case for compound **1**. The sequence of protons was established by a COSY-45° nmr spectrum. The ^{13}C-nmr, broad band, and DEPT spectra showed the presence of seven quaternary, six methine, three methylenes, and two methyl carbons. The proton-carbon (^{1}H-^{13}C) connectivities were established with the help of a HMQC experiment (Table 1). The chemical shifts of various carbon atoms (14) were assigned on the basis of the HMBC spectrum.

The extracts and compounds isolated from the title plant were evaluated for antifungal (16) and antibacterial (17) activities. The petroleum ether extract showed antifungal activity against *Microsporum canis, Pleurotus ostreatus, Alterneria solanii, Curvularia lunata,* and *Epidermophyton floccosum.* Compounds **1** and **2** also showed potent antifungal activity against *E. floccosum* and *P. ostreatus*, and the MIC values for these strains, along with a standard antifungal antibiotic, are shown in Table 2. Neither the petroleum ether extract nor compound **1** exhibited antibacterial activity against *Shigella boydii, Corynebacterium diphtheriae, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis,* or *Salmonella typhi.*

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were recorded on a Varian-MAT 112S mass spectrometer connected to a DEC PDP 11/34 computer system. Hreims were recorded on a JEOL-JMS HX 110 mass spectrometer. ^{1}H-Nmr spectra were recorded on Bruker AM 300 and Bruker AM 500 MHz nmr spectrometers using TMS as internal standard. Ir and uv spectra were recorded on Jasco IRA-1 and Shimadzu UV 240 spectrophotometers, respectively. Tlc was performed on Si gel (GF_{254}) precoated tlc plates (E. Merck and Riedel De Haen).

PLANT MATERIAL.—*Blumea obliqua* (20 kg) was collected in Karachi during May 1991. A voucher specimen was deposited in the herbarium of the Department of Botany, University of Karachi (herbarium No. 63484 KUH).

EXTRACTION AND ISOLATION.—The chopped fresh plant was macerated twice for 10 days with MeOH. The combined extract was filtered and evaporated under reduced pressure to a viscous mass (480 g) that was again extracted with petroleum ether (bp 40–60°). The petroleum ether-soluble portion was evaporated under reduced pressure to an oily paste (57 g) which was chromatographed on a Si gel column and was eluted with hexane, hexane/$CHCl_3$, and $CHCl_3$. The fractions obtained with 10% $CHCl_3$ in hexane were combined and subjected to prep. tlc (Si gel, $CHCl_3$-C_6H_{14}, 1:1) to yield compounds **1** (R_f=0.5) and **2** (R_f=0.6).

5′-Methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene [**1**].—Yellow labile oil (50 mg); uv λ max (Et_2O) 339 nm (ϵ 26,726); ir ν max (CCl_4) 1717 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 0.97 (6H, d, J=6.5 Hz, H-4a), 2.12 (1H, m, H-3a), 2.22 (2H, d, J=6.5 Hz, H-2a), 2.48 (3H, d, J=1.0 Hz, H-6′), 2.78 (2H, t, J=6.7 Hz, H-3″), 4.24 (2H, t, J=6.7 Hz, H-4″), 6.64 (1H, dq, J=3.5 and 1.0 Hz, H-4′), 6.88 (1H, d, J=3.7 Hz, H-3), 6.93 (1H, d, J=3.5 Hz, H-3′), 6.99 (1H, d,

TABLE 2. MIC Values of Compounds **1** and **2** Compared with a Standard Antibiotic.

Organism	**1** (μg/ml)	**2** (μg/ml)	Griseofulvin (μg/ml)
Epidermophyton floccosum	200	200	250
Pleurotus ostreatus	200	200	250

J=3.7 Hz, H-4); ^{13}C-nmr data, see Table 1; eims *m/z* [M]$^+$ 332.0903 (19) ($C_{18}H_{20}O_2S_2$ calcd 332.0904), [M−$C_5H_{10}O_2$]$^+$ 230.0186 (100) ($C_{13}H_{10}S_2$, calcd 230.0223).

5′-Hydroxymethyl-5-[butyl-3-en-1-yn]-2,2′-bithiophene isovaleroxy ester [**2**].—Light yellow labile oil (80 mg); uv λ max (Et_2O) 344 nm (ε 27620); ir ν max (CCl_4) 1725 cm^{-1}; ^{1}H nmr ($CDCl_3$, 500 MHz) δ 0.94 (6H, d, J=7.0 Hz, H-4a), 2.12 (1H, m, H-3a), 2.22 (2H, d, J=7.0 Hz, H-2a), 5.20 (2H, s, H-6′), 5.54 (1H, dd, J=11.0 and 1.0 Hz, H-4″ cis), 5.72 (1H, dd, J=17.5 and 1.5 Hz, H-4″ trans), 6.01 (1H, dd, J=17.5 and 11.0 Hz, H-3″), 6.96 (1H, d, J=3.5 Hz, H-4′), 7.00 (1H, d, J=3.5 Hz, H-3′), 7.01 (1H, d, J=3.5 Hz, H-3), 7.07 (1H, d, J=3.5 Hz, H-4); ^{13}C-nmr data, see Table 1; eims *m/z* [M]$^+$ 330.0750 (38) ($C_{18}H_{18}O_2S_2$, calcd 330.0746), [M−$C_5H_9O_2$]$^-$ 229.0107 (100) ($C_{13}H_9S_2$, calcd 229.0147).

ANTIMICROBIAL ACTIVITY.—Antifungal activity of compounds **1** and **2** was measured by the tube dilution method (16). Compounds were serially diluted in DMSO and added to molten SDA in order to make slants. The slants were inoculated with *Epidermophyton floccosum* and *Pleurotus ostreatus* and incubated at 29° for seven days after which they were observed for inhibition of growth and MIC values were determined. A standard antifungal antibiotic, i.e., griseofulvin was used as standard. The results of antifungal activity in terms of MIC values of compounds **1** and **2** along with the standard are shown in Table 2. Antibacterial activity was performed by the agar well diffusion method (17) using Mueller Hinton Agar medium. The radius of the wells was 5 mm for both 100 μg/100μl and 200 μg/200 μl samples.

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