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Short Communication

Natural Phthalate Derivatives from the Bacterium *Burkholderia cepacia* K87

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Abstract

In continuation of our screening of antifungal active compounds from the fermentation extracts of soil borne bacteria *Burkholderia cepacia* K87 afforded three phthalate derivatives, dibutyl phthalate (**1**), dioctyl phthalate (**2**), and phthalate ester of alkylated 9-hydroxynonanoic acid (**3**). Compound **3** was reported first time. These phthalate derivatives were found to be marginally active or inactive against a number of bacteria and fungi.

Keywords: Burkholderia cepacia K87; Phthalate; Antifungal.

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1. Introduction

The bacterium *Burkholderia cepacia* has been used as a plant-growth promoting rhizobacteria [1, 2]. In continuation of our research for identification of plant growth promoting and antimicrobial compounds from the microbes, we reported two new degraded intermediates of antifungal pyrrolnitrin as 3-chloro-4-(3-chloro-2-nitrophenyl)-5-methoxy-3-pyrrolin-2-one and 4-chloro-3-(3-chloro-2-nitrophenyl)-5-methoxy-3-pyrrolin-2-one from soil bacterium *B. cepacia* K87 [3]. From the methanolic extract of the bacterium, marginally antifungal three phthalate derivatives, dibutyl phthalate (**1**), dioctyl phthalate (**2**), and phthalate ester of alkylated 9-hydroxynonanoic acid (**3**) were isolated. In this paper, we describe the isolation and structure-elucidation of the isolates **1-3**.

2. Materials and Methods

2.1. General methods

High resolution TOF mass spectra (positive ESI mode) were measured on a Waters LCT Premier mass spectrometer coupled with a Waters Alliance HPLC system. Optical

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rotations were measured on a Perkin-Elmer 341-LC Polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Mercury 400 Spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C spectra. The chemical shifts were given in ppm (δ) and were referenced relative to $CDCl_3$ (δ 7.26 and 77.24 ppm for ^{1}H and ^{13}C NMR, respectively). 2D NMR spectra (COSY, HSQC, HMBC and NOESY) were recorded using the manufacturer's software VNMR 6.1C. Flash column chromatography was carried out on a column packed with reversed phase C-18 silica gel (40-63 μm, 90 × 100 mm, Merck). Medium-pressure liquid chromatography (MPLC) was carried out on a Yamazen instrument using a column packed with reversed phase C-18 silica gel (cosmosil 40 C18 Prep, 40 X 320 mm) at 5 mL/min. Preparative HPLC was performed on a Waters 600 model system (Microsorb 100-5 C-18, 21.4 x 250 mm) at 7 mL/min at 210 nm.

2.2. Extraction and isolation

Solid culture media were harvested with MeOH. The methanolic extract (180 g) was suspended in water (100 mL) and subjected to reversed phase C-18 flash column chromatography with a stepwise gradient elution of a mixture of water and MeOH to give eleven fractions (1 L/each fraction). The 90-100% aqueous MeOH eluate (brownish residue, 650 mg) was further chromatographed on a reversed phase C-18 MPLC with a gradient elution of 60% aqueous MeOH to 100% MeOH for 160 min followed by reversed phase HPLC (75%-100% aqueous MeOH for 60 min) to yield compounds **1** (20 mg, R_t = 29.4 min), **2** (7.6 mg, R_t = 33.8 min), and **3** (3.8 mg, R_t = 81.3 min).

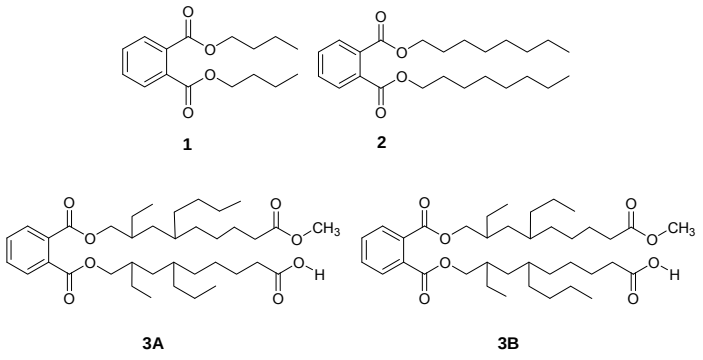

Fig. 1. Structures of the isolated compounds.

Compound 3 [(2-ethyl-4-butyl-8-methoxycarbonyl)octyl (2-ethyl-4-propyl-8-hydroxy carbonyl) octyl phthalate] (3A) *or* ***[(2-ethyl-4-propyl-8-methoxycarbonyl)octyl (2-ethyl-4-butyl-8-hydroxycarbonyl) octyl phthalate] (3B)***: colorless oil; $[\alpha]^{20}_{D}$: -1.49 (*c* 0.28, MeOH); UV (MeOH): λ_{max} *(log* ε*)* 273 (5.79), 225 (6.56) nm; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.70 (2H, *dd*, *J* = 8.8, 2.0 Hz, H-3 and H-6), 7.53 (2H, *dd*, *J* = 8.8, 2.4 Hz, H-4 and H-5), 4.23[a] (2H, *t*, *J* = 6.0 Hz, H-1′), 4.20[a] (2H, *t*, *J* = 6.0 Hz, H-1″), 3.66 (3H, *s*,

OC*H*$_3$, H-10′), 2.30^b (2H, *t*, *J* = 7.2 Hz, H-8′), 2.35^b (2H, *t*, *J* = 8.0 Hz, H-8″), 1.68 (2H, *m*, H-2′and H-2″), 1.62 (4H, *m*, H-7′ and H-7″), 1.13-1.43 (28H, *m*, H-3′, H-4′, H-5′, H-6′, H-11′, H-13′, H-14′, H-15′, H-3″, H-4″, H-5″, H-6″, H-10″, H-12″ and H-13″), 0.92 (3H, *t*, *J* = 7.2 Hz, H-12′), 0.90 (3H, *t*, *J* = 7.2 Hz, H-11″), 0.88 (3H, *t*, *J* = 7.2 Hz, H-16′), 0.64 (3H, *t*, *J* = 7.2 Hz, H-14″) (values with same superscripts are interchangeable). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 179.0 (C-9″), 174.0 (C-9′), 167.0 (C-1, C-8), 132.5 (C-2 and C-7), 130.9 (C-4 and C-5), 128.9 (C-3 and C-6), 68.4 (C-1′ and C-1″), 51.7 (O*C*H$_3$), 39.0 (C-2′ and C-2″), 34.5 (C-8″), 34.0 (C-8′), 32.3 (C-13′), 30.5^a (C-3′), 30.7^a (C-3″), 30.0^b (C-5′), 29.9^b (C-5″), 29.7 (C-4′), 29.6 (C-4″), 29.5^c (C-6′), 29.4^c (C-6″), 29.3 (C-12″), 29.0 (C-14′), 25.3^d (C-6′), 25.1^d (C-6″), 24.1 (C-11′and C-10″), 23.0 (C-15′ and C-13″), 16.1 (C-14″), 14.5^e (C-12′), 14.4^e (C-16′ and C-11″) (values with same superscripts are interchangeable). HRTOFMS: [M+H] *m/z* 647.4537 (calcd. for $C_{38}H_{63}O_8$ + H, 647.4523); TLC (hexane-EtOAc 95:5) R_f 0.21.

2.3. Antimicrobial and cytotoxicity assays

Antimicrobial and cytotoxicity assays were carried out according to the methods described in the literatures [3, 17, 18].

4. Results and Discussion

The broth of *B. cepacia* K87 (10 L) was extracted with MeOH [3]. The methanolic extract was subjected to a series of chromatography (reversed-phase C-18 flash column chromatography followed by C-18 MPLC and HPLC). Compounds, **1**, **2**, and **3** were obtained in yield of 2.8, 20, and 7.6 mg, respectively. Compounds **1** and **2** were isolated as colorless oil and established as dibutyl phthalate, and dioctyl phthalate, respectively [4, 5]. Compound **3** was obtained as colorless oil with specific rotation of -1.49 (*c* 0.28, MeOH). The molecular formula was determined to be $C_{38}H_{63}O_8$ + H, 647.4537 by HRTOFMS ([M+H] *m/z* (calcd. for $C_{38}H_{63}O_8$ + H, 647.4523). The ^{1}H NMR spectra showed the signals for phthalate moiety: δ_H 7.70 (dd, *J* = 8.8, 2.0 Hz) and δ_C 128.9; δ_H 7.53 (dd, *J* = 8.8, 2.4 Hz) and δ_C 130.9; and the quarternary carbon at δ_C 132.5. From the interpretations of ^{1}H-^{1}H COSY the substructures of **3** were deduced to be -O-CH_2-CH<, CH_3CH_2-CH<, >CH-CH_2-CH<, $CH_3CH_2CH_2CH_2CH$<, $CH_3CH_2CH_2CH$<, and CH_3-CH_2-(CH_2)n- moieties, where the methine groups might be repeated due to severe overlapping of the signals. From interpretation of HSQC and HMBC (Fig. 2) spectral data, the substructures were connected to provide two alkylated 9-hydroxynonanoate moiety which were ester-linked with the phthalate moiety as evidenced from the correlations between the carbonyl groups at δ167.0 (2C) and the hydroxylated methine protons at δ 4.23 and 4.20. The methoxy group at δ_H 3.66 was correlated with one of the carbonyl groups of the nonanate at δ_C 174.0. The numbers of severely overlapped methylene and methine groups were counted from the molecular formula deduced from the MS data. Due to severe overlapping the propyl and butyl groups could be interchangeable between the chains. Thus, we suggest

the structure of **3** could be either [2-ethyl-4-butyl-8-methoxycarbonyl]octyl [2-ethyl-4-propyl-8-hydroxycarbonyl]octyl phthalate (**3A**) or [2-ethyl-4-propyl-8-methoxycarbonyl]octyl [2-ethyl-4-butyl-8-hydroxycarbonyl]octyl phthalate (**3B**).

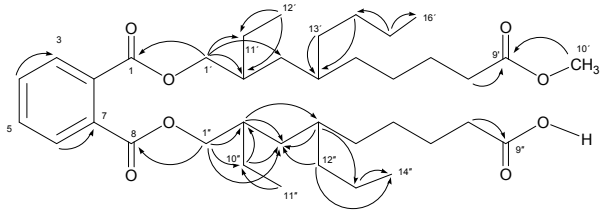

Fig. 2. Important HMBC correlations in **3A** (H to C).

Compounds **1**-**3** were tested against the bacterial strains like *Streptococcus mutans*, *Bacillus subtilis*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and the fungal strains like *Candida kruisii, Candida glabrata*, *Candida albicans*, and *Rhizoctonia solani.* Compounds **1** and **2** were found to be inactive against all the microbial strains tested at 100 µg. Compound **3** possessed marginal antifungal activity only against *C. kruisii* (zone of inhibition 9 mm at 200 µg) but inactive against other bacteria.

Synthetic phthalate esters are used commonly in paints and polymer products as plasticizers [6, 7], and widely detected in the environments, such as sediments, natural waters, soils, plants, and aquatic organisms [8]. Although phthalate derivatives are useful chemicals, they are regarded as environmental health hazards due to their toxicity, carcinogenicity, teratogenicity, and mutagenicity [7, 9-11]. It was reported that prenatal exposures of animal to some phthalates, particularly di(2-ethylhexyl) phthalate, dibutyl phthalate, and benzyl butyl phthalate caused male reproductive anomalies including decreased anogenital distance, testicular dysgenesis, hypospadias, cryptorchidism, malformations of the male reproductive organs, somniferous tubule degeneration [12-14]. Phthalate exposure in childhood has also caused an increased occurrence of atopic disease including allergic rhinitis, wheezing, and eczema [9, 15]. However, there are reports that phthalate esters are naturally produced extracellularly by microorganisms such as bacteria, fungi and yeasts [4, 6, 16]. Compounds **1-3** were isolated from the extract of the culture broth of plant-growth promoting rhizobacteria *B. cepacia* K87. It is unclear whether they were true metabolites of the bacterium or artifacts formed during isolation.

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