



Pergamon

Tetrahedron Letters 41 (2000) 1657–1660

TETRAHEDRON  
LETTERS

## Cordyanhydrides A and B. Two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620

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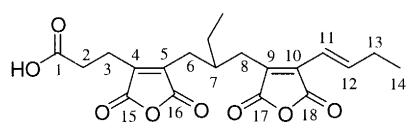
Received 9 November 1999; accepted 21 December 1999

### Abstract

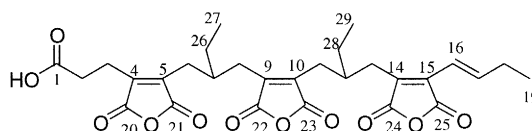
Cordyanhydrides A and B, two new alkenoic acids bearing two and three maleic anhydride moieties in the linear acid chain, were isolated and identified from a culture broth of the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Cordyceps pseudomilitaris*; insect pathogenic fungi; anhydride; nonadride.

*Cordyceps pseudomilitaris* Hywel-Jones is an insect pathogenic fungus that infects the immature stage of the order Lepidoptera.<sup>1</sup> The morphology of this fungus is closely related to that of *C. militaris* which is known to produce several secondary metabolites including a nucleoside antibiotic, cordycepin.<sup>2</sup> In a course of our search on novel natural products from insect pathogenic fungi, we had undertaken the chemical investigations of the strain *C. pseudomilitaris* BCC 1620. Two new alkenoic acids **1** and **2**, bearing two and three maleic anhydride moieties in the linear chain, were isolated and named cordyanhydrides A and B, respectively. Herein we report the method of isolation and structure elucidation of these compounds with hitherto unknown skeletons.



**1**



**2**

Strain BCC 1620 was collected from Sam Lan National Park, central Thailand, identified by Dr. Nigel L. Hywel-Jones, and deposited at the BIOTEC culture collection. The isolated strain was grown on potato dextrose agar at 22°C for 14 days before inoculation as several pieces of culture (5×5 mm) into 8×1 L Erlenmeyer flasks, each containing 250 mL of potato dextrose broth. The cultures were incubated on a rotary shaker (180 rpm) at 22°C for 28 days. The culture filtrate (ca. 2 L) was extracted with EtOAc (2×2

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Table 1  
 $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data for compound **1**

position	$\delta_{\text{C}}$ (Mult.)	$\delta_{\text{H}}$ (Mult., J Hz)	position	$\delta_{\text{C}}$ (Mult.)	$\delta_{\text{H}}$ (Mult., J Hz)
1	176.8 (s)	7.9 (br) <sup>a</sup>	11	115.8 (d)	6.22 (dt, 15.8, 1.6)
2	30.7 (t)	2.78 (m)	12	150.6 (d)	7.29 (dt, 15.7, 6.7)
3	19.8 (t)	2.78 (m)	13	27.4 (t)	2.33 (m)
4	143.4 (s)	-	14	12.4 (q)	1.12 (7.5)
5	144.2 (s)	-	15	165.1 (s)	-
6	28.7 (t)	2.58 (m), 2.45 (m)	16	165.5 (s)	-
7	38.2 (d)	2.21 (m)	17	166.2 (s)	-
8	28.2 (t)	2.55 (m), 2.41 (m)	18	164.0 (s)	-
9	136.7 (s)	-	19	26.6 (t)	1.34 (m)
10	138.5 (s)	-	20	10.7 (q)	0.98 (t, 7.3)

<sup>a</sup>Carboxylic proton.

L) to obtain a yellow gum. The crude extract was passed through a Sephadex LH-20 column with MeOH as eluent, and further purified by preparative HPLC, using a reversed-phase column (Prep Nova-Pak HR C<sub>18</sub>, 40×100 mm) with MeCN:H<sub>2</sub>O 60:40 as eluent, to obtain **1** (21 mg) and **2** (29 mg).

The molecular formula of cordyanhydride A (**1**), C<sub>20</sub>H<sub>22</sub>O<sub>8</sub> was determined by HR-FABMS<sup>3</sup> and  $^{13}\text{C}$  NMR (Table 1). The  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR,  $^1\text{H}$ - $^1\text{H}$  COSY and HMQC spectra of **1** indicated that the compound possesses three structural fragments: -CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>CH(CH<sub>2</sub>CH<sub>3</sub>)CH<sub>2</sub>-, and a *trans*-1-butenyl group. In addition to proton signals due to these fragments, a carboxyl proton at  $\delta_{\text{H}}$  7.9 (br, 1H, exchangeable with D<sub>2</sub>O) was detected. The  $^{13}\text{C}$  NMR and DEPT spectra revealed the presence of five carbonyl carbons at  $\delta_{\text{C}}$  176.8, 166.2, 165.5, 165.1 and 164.0, and four quaternary carbon signals due to two tetrasubstituted olefins at  $\delta_{\text{C}}$  144.2, 143.4, 138.5 and 136.7. Analysis of HMBC correlations established the connectivity of the three structural fragments and the nine quaternary carbons. Representative long range correlations of quaternary carbons are shown in Fig. 1. From the molecular formula of **1**, formation of two acid anhydrides was indicated. The FT-IR spectrum of **1**, with absorptions at  $\nu$  1825 (C=O stretching), 1765 (s, br, C=O stretching) and 920 (C-O stretching) cm<sup>-1</sup> were consistent with the structure bearing maleic anhydride moieties [2,3-dimethyl maleic anhydride:  $\nu$  1850, 1750, 915 cm<sup>-1</sup>]. An additional carbonyl absorption at  $\nu$  1715 and a broad band at  $\nu$  3400 to 2000 were in good agreement with the presence of an additional carboxyl group.

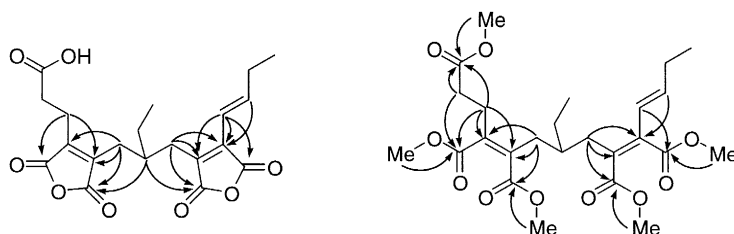
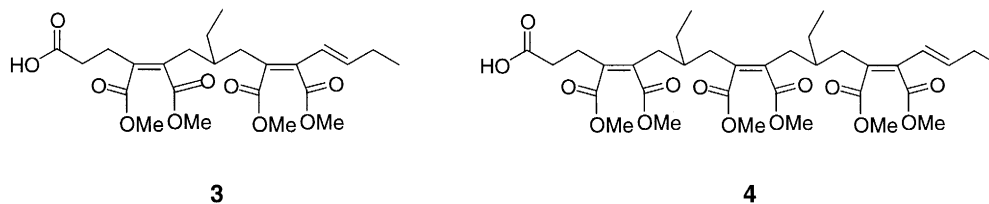


Fig. 1. HMBC correlations of quaternary carbons found for **1** and **3**

Cordyanhydride B (**2**), with molecular formula C<sub>29</sub>H<sub>32</sub>O<sub>11</sub> (elemental analysis,  $^1\text{H}$  and  $^{13}\text{C}$  NMR),<sup>4</sup> showed a similar FT-IR spectrum to that of **1**. NMR analysis ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPTs,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and

HMBC) revealed the presence of an additional  $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}_2-$  fragment and a maleic anhydride unit. Thus, a structure with repetition of these structural fragments in the linear chain of **1** was consistent with the spectral data.

Structures of **1** and **2** were further confirmed by one-pot conversions into corresponding penta- and heptamethyl esters **3** and **4**, respectively. Hydrolysis of **1** in aq.  $\text{K}_2\text{CO}_3/\text{MeOH}$  (rt, 6 h), followed by methylation with excess  $\text{MeI}/\text{K}_2\text{CO}_3$  in DMF (rt, 4 days) gave **3** (40% yield, after purification by silica gel column chromatography).<sup>5</sup> In the same fashion, compound **4** was obtained from **2** (71%).<sup>6</sup> Unfortunately, we were unable to solve the absolute configurations of C(7) and C(12) of compound **2**, as well as C(7) of compound **1**. According to the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound **2**, the purified sample is a single isomer, and the other diastereomer has not been detected from the culture of the strain BCC 1620.



Compounds bearing acid anhydride moieties are rare in nature. Cordyanhydrides A and B belong to the nonadrides family which include formal  $\text{C}_9$ -units represented as 2-(1-butenyl)-3-methylmaleic anhydrides.<sup>7,8</sup> Most of the previously reported nonadrides contain either one<sup>9</sup> or two  $\text{C}_9$ -units, where these units are connected by the head to head or head to tail coupling to furnish a nine-membered ring.<sup>10</sup> To our knowledge, compound **2** is the first occurrence of a nonadride containing three  $\text{C}_9$ -units. In addition, these two and three  $\text{C}_9$ -units are linearly connected in compounds **1** and **2**, a novel structure for the nonadrides family.

## Acknowledgements

Financial support by the Biodiversity Research and Training Program (BRT) is gratefully acknowledged. We are also grateful to the fermentation technology laboratory of the BIOTEC for mass cultivation of the fungus, and to Professor Eiichi Nakamura and Mr. Go Sakata, The University of Tokyo, for a HRMS measurement. One of us (Y.T.) thanks the National Science and Technology Development Agency (NSTDA) for the Senior Research Fellowship Award.

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- Compound **1**: colorless oil;  $[\alpha]_D^{26} -1^\circ$  (c 0.94,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 209 (4.26), 262 (3.98), 3.11 (3.85); HR-FABMS  $m/z$  391.1404 (calcd for  $\text{C}_{20}\text{H}_{23}\text{O}_8$   $[\text{M}+\text{H}]^+$  391.1393); EIMS  $m/z$  390  $[\text{M}]^+$ , 373, 372, 207, 166.
- Compound **2**: colorless amorphous solid;  $[\alpha]_D^{25} +1^\circ$  (c 1.15,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 209 (4.31), 262 (4.20); IR (KBr)  $\nu_{\text{max}}$  1825, 1765 (s), 1715, 1650, 1275, 1225, 925, 750  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.9 (1H, brs,  $-\text{CO}_2\text{H}$ ), 7.28 (1H, dt,  $J=15.8, 6.7$  Hz, H-17), 6.23 (1H, d,  $J=15.8$  Hz, H-16), 2.77 (4H, m, H-2 and H-3), 2.58–2.53 (4H, m, H-6a, H-8a, H-11a and H-13a), 2.42–2.39 (4H, m, H-6b, H-8b, H-11b and H-13b), 2.35 (1H, m, H-18), 2.24 (2H, m, H-7 and H-12), 1.34 (4H, m, H-26 and H-18), 1.12 (3H, t,  $J=7.4$  Hz, H-19), 0.97 (6H, t,  $J=7.3$  Hz, H-27 and H-29);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  176.8 (s, C-1), 166.3 (s, C-24), 165.6 (s), 165.5 (s), 165.5 (s), 165.0 (s), 164.0 (s, C-25), 150.6 (d, C-17), 144.3 (s), 144.2 (s), 144.0 (s), 143.6 (s), 138.5 (s, C-15), 136.6 (s, C-14), 115.9 (d, C-16), 37.9 (d), 37.7 (d), 30.6 (t, C-2), 28.9 (t),

- 28.8 (t), 28.6 (t), 28.1 (t, C-13), 27.4 (t, C-18), 26.7 (t), 26.6 (t), 19.7 (t, C-3), 12.3 (q, C-19), 10.6 (q, C-27), 10.6 (q, C-29). Anal. C 62.59%, H 5.83%, calcd for  $C_{29}H_{32}O_{11}$ , C 62.58%, H 5.80%.
5. Compound **3**: colorless oil; IR (KBr)  $\nu_{\max}$  1732 (br), 1635, 1436, 757  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.31 (1H, dt,  $J=15.8$ , 1.6 Hz, H-11), 6.01 (1H, dt,  $J=15.8$ , 6.6 Hz, H-12), 3.84 (3H, s, 18- $\text{OCH}_3$ ), 3.75 (3H, s, 15- $\text{OCH}_3$ ), 3.73 (3H, s, 16- $\text{OCH}_3$ ), 3.72 (3H, s, 17- $\text{OCH}_3$ ), 3.68 (3H, s, 1- $\text{OCH}_3$ ), 2.69 (2H, m, H-3), 2.45 (2H, m, H-2), 2.44–2.42 (2H, m, H-6a and H-8a), 2.37 (1H, dd,  $J=13.7$ , 6.5 Hz, H-6b), 2.31 (1H, dd,  $J=14.0$ , 6.9 Hz, H-8b), 2.24 (2H, m, H-13), 1.63 (1H, m, H-7), 1.31 (2H, m, H-19), 1.05 (3H, t,  $J=7.5$  Hz, H-14), 0.91 (3H, t,  $J=7.3$  Hz, H-20);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  172.5 (s, C-1), 169.3 (s, C-18), 168.8 (s, C-17), 168.5 (s, C-15), 167.5 (s, C-16), 143.4 (d, C-12), 142.8 (s, C-10), 138.2 (s, C-5), 52.3 (q, 17- $\text{OCH}_3$ ), 52.3 (q, 18- $\text{OCH}_3$ ), 52.2 (q, 15- $\text{OCH}_3$ ), 52.2 (q, 16- $\text{OCH}_3$ ), 51.8 (q, 1- $\text{OCH}_3$ ), 39.6 (d, C-7), 33.5 (t, C-8), 32.1 (t, C-2), 31.2 (t, C-6), 26.6 (t, C-13), 26.0 (t, C-19), 25.0 (t, C-3), 12.9 (q, C-14), 11.1 (q, C-20). Anal. C 60.42%, H 7.33%, calcd for  $C_{25}H_{36}O_{10}$ , C 60.47%, H 7.31%.
6. Compound **4**: colorless oil; IR (KBr)  $\nu_{\max}$  1732 (br), 1636, 1437, 792  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.31 (1H, d,  $J=15.8$  Hz, H-16), 6.01 (1H, dt,  $J=15.7$ , 6.5 Hz, H-17), 3.75 (3H, s), 3.74–3.73 (12H, m), 3.71 (3H, s), 3.68 (3H, s, 1- $\text{OCH}_3$ ), 2.68 (2H, t,  $J=7.7$  Hz, H-3), 2.44 (2H, t,  $J=7.7$  Hz, H-2), 2.40–2.25 (8H, m, H-6, H-8, H-11 and H-13), 2.24 (2H, m, H-18), 1.54 (2H, m, H-7 and H-12), 1.29 (4H, m, H-26 and H-28), 1.03 (3H, t,  $J=7.5$  Hz, H-19), 0.90 (3H, t,  $J=7.3$  Hz, H-29), 0.88 (3H, t,  $J=7.5$  Hz, H-27);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  172.5 (s, C-1), 169.2 (s), 169.1 (s), 168.9 (s), 168.7 (s), 168.1 (s, C-20), 167.4 (s), 143.3 (d, C-17), 142.7 (s, C-15), 139.0 (s), 138.9 (s), 137.1 (s), 136.0 (s), 127.3 (s, C-14), 122.6 (d, C-16), 52.2 (q), 52.2 (q), 52.1 (q), 52.1 (q), 52.0 (q), 52.0 (q), 51.7 (q, 1- $\text{OCH}_3$ ), 39.8 (d), 38.0 (d), 33.9 (t), 33.6 (t), 33.3 (t), 32.1 (t, C-2), 31.0 (t), 26.5 (t, C-18), 25.7 (t), 25.6 (t), 24.6 (t, C-3), 12.9 (q, C-19), 11.0 (q), 10.9 (q). Anal. C 61.04%, H 7.39%, calcd for  $C_{36}H_{52}O_{14}$ , C 61.00%, H 7.39%.
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