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Tartrolone C: A Novel Insecticidal Macrodiolide Produced by *Streptomyces* sp. CP1130

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A new member of the tartrolone series of macrodiolides, tartrolone C (**1**), was isolated from a *Streptomyces* species on the basis of its insecticidal activity. Metacycloprodigiosin (**2**) and undecylprodigiosin (**3**) were also isolated on the same basis. The structure of all compounds was established by spectroscopic data (NMR, MS, and UV).

Natural products have had demonstrated commercial impact in the agrochemical arena, both as leads to new modes of action and as products in their own right. Examples include the synthetic strobilurins, structurally derived from the fungicidal natural products strobilurin A and oudemansin A,[1,2] and the spinosyn class[3] of potent insecticides.

During Dow AgroSciences' screening program to find novel natural products with agrochemically relevant biological activity, an extract of *Streptomyces* strain CP1130 exhibited good levels of activity in a beet army worm (BAW) assay. Subsequent reversed-phase LC-MS analysis with BAW bioassay of the eluent showed two classes of lipophilic insecticidal metabolites. One bioactive region contained a single component with λ_{max} = 230 nm and a nominal molecular weight of 888, showing several losses of 18 amu, indicating multiple hydroxylation sites. There were no good matches for this material with compounds either in our internal or in commercially available databases. The remaining active region contained several related components, the major one of which had λ_{max} = 470 nm, accounting for the deep purple pigmentation of the culture, and a molecular weight of 391, suggesting it to be a member of the prodigiosin class.

Preliminary experiments on the organism grown in liquid culture showed that neither of these metabolite classes was excreted into the growth medium to any significant extent; therefore, subsequent isolation focused on methanol extracts of the cell pellet. The methanolic cell extract was concentrated to an aqueous phase and partitioned with CH_2Cl_2, and the components in the organic phase were separated using semipreparative C_8 HPLC. This afforded the novel tartrolone C (**1**) and the known compounds metacycloprodigiosin (**2**) and undecylprodigiosin (**3**), identified by comparison of their UV, NMR, and MS data with literature data.[4,5]

LC-MS of **1** showed +ESI ions at 888.5 (20% relative abundance) and 889.5 ($[M + H]^+$; 80% relative abundance), consistent with the presence of a boron atom in the molecule and with a molecular weight of 888.5 for the conjugate acid ($C_{46}H_{68}{}^{11}BO_{16}H$). This was further supported by the +ESI $[M + NH_4]^+$ and −ESI $[M - H]^-$ ion clusters, which showed the expected range of ions. High-resolution LC-MS on the ammonium and sodium adducts gave masses of 906.5004 and 911.4584, within −1.8 and +0.8 mmu, respectively, of the calculated values for the adducts of the ^{11}B-conjugate acid, providing additional support for structure **1**.

Table 1. NMR Data for Compound **1**

atom	δ_H, mult (J)[a]	δ_C, (mult)[b]	HMBC[c]
1		173.4 (s)	
2	4.80, s	77.6 (d)	1, 3, 4
3		104.7 (s)	
4		71.5 (s)	
4-OH	3.27, s		3, 4, 5, 22
5	1.99, m	33.7 (t)	
	1.65, m		
6	1.58, m	24.8 (t)	
	1.47, m		
7	4.18, ddt (11.3, 11.3, 2.6)	70.3 (d)	8, 9
8	2.26, dq (11.3, 7.2)	54.3 (d)	7, 9, 23
9		216.7 (s)	
10	3.02, dd (18.9, 10.8)	41.4 (t)	9, 11, 12
	2.66, d (18.4)		9
11	3.94, ddd (9.7, 9.7, 3.6)	68.7 (d)	9, 13
11-OH	4.23, s		11, 12
12	1.67, m	32.2 (t)	
	1.43, m		
13	2.38, m	28.0 (t)	
	2.03, m		
14	5.90, dt (15.4, 5.1)	135.2 (d)	
15	5.98, dd (15.4, 10.8)	122.6 (d)	
16	6.09, dd (10.2, 10.2)	131.2 (d)	
17	5.21, ddd (1.3, 11.3, 5.6)	127.7 (d)	
18	2.42, m	23.2 (t)	
	2.96, m		
19	1.85, m	35.3 (t)	
	1.40, m		
20	4.75, dq (6.7, 5.6)	70.5 (d)	1, 18, 19, 21
21	1.24, d (6.7)	20.3 (q)	19, 20
22	1.33, s	25.0 (q)	3, 4, 5
23	1.03, d (7.2)	13.6 (q)	7, 8, 9

[a] Recorded in $CDCl_3$ at 600 MHz, J values in Hz. [b] Recorded in $CDCl_3$ at 150 MHz. [c] Key correlations from experiment were optimized for 8 Hz coupling.

The NMR data and assignments for metabolite **1** are listed in Table 1. It became quickly apparent from the MS and ^{1}H NMR data that the molecule was a symmetrical dimer, similar to the known boron-containing tartrolone B,[6−9] but containing an extra oxygen atom in each monomeric subunit. Proton resonances and spin systems were assigned by analysis of COSY and HSQC experiments, and these spin-systems were readily connected by analysis of an HMBC experiment. The key difference was the substitution of a methyl doublet in tartrolone B with a singlet in **1**. HMBC correlations from this singlet were shown to both a doubly oxygenated and a singly oxygenated carbon atom, as well as to the methylene C-5 (Figure 1). Additionally,

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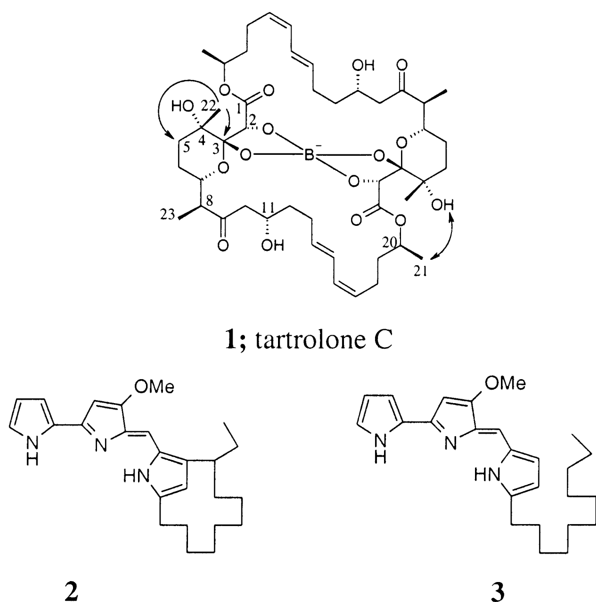

Figure 1. Compounds isolated as described in the text. For tartrolone C, key NOE (double headed) and HMBC correlations (single headed) are shown.

these two oxygenated carbon atoms showed HMBC cross-peaks from 2-H, indicating that the additional oxygen atom was located on C-4 of the tartrolone monomeric unit.

NOE experiments indicated that the relative stereochemistry of tartrolone C was the same as that for tartrolone B,[6] for which a detailed NMR analysis has been published.[7] In addition to the ^{1}H spectrum being very similar to the published spectrum of tartrolone B, results from 1D NOESY experiments on **1** were in total agreement with distance measurements obtained from the X-ray structure for tartrolone B. Additionally, an enhancement of the hydroxyl proton (4-OH) was seen on irradiation of the methyl group 21-H, indicating the stereochemistry of C-4 as shown. Finally, the optical rotation was of the same sign and very close in magnitude to that of tartrolone B,[7] whose absolute stereochemistry was previously established from X-ray data. Thus we were able to assign the stereochemistry for tartrolone C as drawn (**1**).

Tartrolone C was active on BAW and tobacco bud worm (TBW), with MELCs[10] of 125 ppm on both insects, approximately 40× and 310× *less* active than a standard of spinosyn A (MELC = 3 ppm on BAW and 0.4 on TBW).

A partial 16S ribosomal-RNA sequence of CP1130 matched the genus *Streptomyces*, most closely the species *verticillus*. Therefore, this represents the first report of a tartrolone from a *Streptomyces* species, since their only previously reported occurrence was in *Sorangium cellulosum*, a myxobacterium.[7] The similar boromycin[11,12] and aplasmomycin[13] families, while from *Streptomyces* species, are structurally more distant from the tartrolones.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on a Bruker DRX600 instrument operating at 600.13 MHz (^{1}H) and 150.62 MHz (^{13}C). All 2D experiments were acquired using standard Bruker pulse sequences supplied with Xwinnmr using gradient selection where possible. Nuclear Overhauser effect (NOE) experiments were carried out using a 1D NOESY sequence utilizing gradient selection.[14] Optical rotation was determined on a Perkin-Elmer model 241 polarimeter. LC-MS was performed on a Micromass Platform single-quadrupole mass spectrometer fitted with a Z-Spray LC-MS source. Elution was performed on a Hypersil-C_8-BDS column (250 × 4.6 mm; 5 μm) with a linear gradient from 100% 10 mM ammonium acetate at pH 6.0 to 100% acetonitrile over 20 min, using a Hewlett-Packard 1100 LC system. This system was equipped with UV-diode array detection, from which UV λ_{max} values were obtained. Accurate LC-MS and LC-MS/MS experiments were conducted on a Micromass hybrid quadrupole-time-of-flight (Q-Tof) instrument.

Semipreparative LC was performed using a Hewlett-Packard 1050 LC system with UV-diode array detection. Components of interest were separated using a HS-Hyperprep-C_8-BDS column (250 × 10 mm), eluted using 10 mM NH_4OAc–MeCN (20:80) at 5 mL/min.

Bacterial Strain. CP1130 was isolated from a soil sample from Curaçao. It was maintained on AS1 agar composed of 1.0 g of yeast extract, 0.2 g of L-alanine, 0.2 g of L-arginine, 0.2 g of asparagine, 5.0 g of soluble starch, 2.5 g of NaCl, 10.0 g of $NaSO_4$, 15.0 g of Bacto agar, and 1 L of Milli-Q water. Colonies from freshly sporulated agar cultures were transferred to four tribaffled 250 mL flasks each containing 50 mL of vegetative medium. The half-strength N-Z Amine seed medium was composed of 5.0 g of glucose, 10.0 g of soluble starch, 2.5 g of yeast extract, 2.5 g of N-Z Amine Type A (Sigma), 0.5 reagent grade $CaCO_3$, and 1 L of Milli-Q water. The vegetative cultures were incubated at 30 °C on a New Brunswick G-25 rotary shaker at 150 rpm for 3 days. Scale-up cultures were then produced from these vegetative cultures on a 3 L scale. Six 2800 mL tribaffled wide-mouth Fernbach flasks containing 500 mL of CSI growth medium were inoculated with 5 mL of 3-day-old vegetative culture. The CSI medium contained 15 g of Nutrisoy grits, 1 g of casein, 25 g of cerelose, 3 g of blackstrap molasses, 2.5 g of $CaCO_3$, 2 mL of Czapek's mineral mix, and 1 L of Milli-Q water. Czapek's mineral mix contained 100 g of KCl, 100 g of $MgSO_4\cdot 7H_2O$, and 2 g of $FeSO_4\cdot 7H_2O$. The Fernbach flasks were incubated on a New Brunswick G-25 rotary shaker at 150 rpm for 7 days at 30 °C. The cultures, which were deep pink in color and thick with growth, were harvested and extracted as described below. A sample of strain CP1130 is deposited at Dow AgroSciences (9330 Zionsville Rd., Indianapolis, IN 46268).

Bioassay Conditions. Lepidopteran diet was dispensed into the wells of a 96-well microtiter plate (100 μL/well) and allowed to cool and dry. Test samples were dried in the wells of a second 96-well plate, then dissolved in acetone–water (50:50; 50 μL) with sonication. The test sample solutions were transferred to the plate containing insect diet and allowed to dry on the surface of the diet. Each well was then infested with either 8–10 beet armyworm (BAW) eggs or 4 or 5 tobacco budworm (TBW) eggs. Test plates were covered with a layer of sterile cotton and then the plate lid. The effects of the test compounds on the development of the insects were evaluated after a 6 or 7 day incubation period at 28 °C.

Extraction and Isolation. The culture broth was centrifuged to harvest the cells, which were extracted with MeOH (2 × 750 mL; orbital shaking for 1.5 h each time), giving extracts CX-1 and CX-2. Cell extract CX-1 was concentrated under nitrogen, and the orange pigment that precipitated was filtered off and discarded. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL), which was dried in vacuo to give a red solid (29 mg). The components in this extract were separated by semipreparative HPLC to give **1** (1.3 mg) and **2** (0.9 mg).

Cell extract CX-2 was dried, and the red solid residue was triturated using MeOH. The soluble phase was dried on an aliquot of silica gel and chromatographed over silica gel using a gradient of MeOH–acetic acid (99:1) in DCM–acetic acid (99:1). The biologically active fractions were pooled and dried, giving a dark red solid (188 mg). An aliquot of this solid (25 mg) was dissolved in MeOH, and the components were separated by semipreparative HPLC to give additional **1** (1.5 mg) and **2** (9.5 mg) plus **3** (2.3 mg).

Tartrolone C (1): white solid; $[\alpha]^{25}_{D}$ +34.7° (*c* 0.2, $CHCl_3$); UV (PDA detector of HPLC) λ_{max} 230 nm, ^{1}H NMR and ^{13}C NMR, see Table 1; low-resolution MS +ESI *m/z* 888.5 ([M +

H]$^+$ for $C_{46}H_{68}{}^{10}BO_{16}H$; 20% of parent ion peak), *m*/*z* 889.5 ([M + H]$^+$ for $C_{46}H_{68}{}^{11}BO_{16}H$; 80%), *m*/*z* 905.5 ([M + NH_4]$^+$ for $C_{46}H_{68}{}^{10}BO_{16}H$; 20% of base peak), *m*/*z* 906.5 ([M + NH_4]$^+$ for $C_{46}H_{68}{}^{11}BO_{16}H$; 80%); −ESI *m*/*z* 886.5 ([M − H]$^-$ for $C_{46}H_{68}$-$^{10}BO_{16}H$; 20% of base peak), *m*/*z* 887.5 ([M − H]$^-$ for $C_{46}H_{68}$-$^{11}BO_{16}H$; 80%); HRMS *m*/*z* 906.5004 ± 0.0030 (calcd for $[C_{46}H_{68}{}^{11}BO_{16}H + NH_4]^+$, 906.5022, Δ = −1.8 mmu), *m*/*z* 911.4584 ± 0.0039 (calcd for $[C_{46}H_{68}{}^{11}BO_{16}H + Na]^+$, 906.4576, Δ = +0.8 mmu).

References and Notes

(1) Anke, T.; Hecht, H. J.; Schramm, G.; Steglich, W. *J. Antibiot.* **1979**, *32*, 1112−1117.
(2) Clough, J. M.; De Fraine, P. J.; Fraser, T. E. M.; Godfrey, C. R. A. *ACS Symp Ser.* **1992**, *504* (Synth. Chem. Agrochem. III), 372−383.
(3) Sparks, T. C.; Crouse, G. D.; Durst, G. *Pest Manage. Sci.* **2001**, *57*, 896−905.
(4) Wasserman, H. H.; Keith, D. D.; Rodgers, G. C. *Tetrahedron* **1976**, *32*, 1855−1861.
(5) D'Alessio, R.; Bargiotti, A.; Carlini, O.; Colotta, F.; Ferrari, M.; Gnocchi, P.; Isetta, A.; Mongelli, N.; Motta, P.; Rossi, A.; Rossi, M.; Tibolla, M.; Vanotti, E. *J. Med. Chem.* **2000**, *43*, 2557−2565.
(6) Schummer, D.; Schomburg, D.; Irschick, H.; Reichenbach, H.; Hoefle, G. *Liebigs Annalen.* **1996**, *6*, 965−969.
(7) Schummer, D.; Irschick, H.; Reichenbach, H.; Hoefle, G. *Liebigs Ann. Chem.* **1994**, *3*, 283−289.
(8) Reichenbach, H.; Hoefle, G.; Irschick, H.; Schummer, D. German Patent Application 93-4313990 930428.
(9) Irschick, H.; Schummer, D.; Gerth, K.; Hoefle, G.; Reichenbach, H. *J. Antibiot.* **1995**, *48*, 26−30.
(10) MELC = minimum emergent larvicide concentration; this is the minimum amount of compound showing an emergent larvicide behavior in the lepidopteran diet assays.
(11) Kohno, J.; Kawahata, T.; Otake, T.; Morimoto, M.; Mori, H.; Ueba, N.; Nishio, M.; Kinumaki, A.; Komatsubara, S.; Kawashima, K. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1036−1037.
(12) Pache, W.; Zaehner, H. *Arch. Mikrobiol.* **1969**, *67*, 156−65.
(13) Aplasmomycins as Acaricides and Insecticides. Hokko Chemical Industry Co., Ltd., Japanese Patent 57056407.
(14) Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302−324.

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