Prunolides A, B, and C: Novel Tetraphenolic Bis-Spiroketals from the Australian Ascidian *Synoicum prunum*

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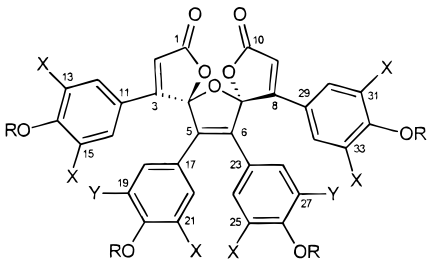
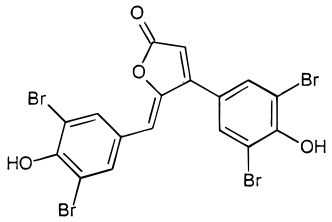
Received September 15, 1998 (Revised Manuscript Received January 25, 1999)

Three novel tetraphenolic bis-spiroketals, prunolides A−C (**1**, **3**, and **4**) have been isolated from the Australian ascidian *Synoicum prunum*. The structures were determined from NMR spectroscopic data and from an X-ray analysis of prunolide A. The prunolides contain a unique 1,6,8-trioxadispiro-[4.1.4.2]trideca-3,10,12-triene-2,9-dione carbon skeleton. The known compound rubrolide A (**5**) was also isolated.

Ascidians have been the focus of intensive chemical investigation in recent years since they have proven to be rich sources of unique and often biologically active compounds.[1] A noteworthy example is ecteinascidin 743 which possesses a unique skeleton and is currently in phase 1 clinical trials as an anticancer agent.[2] As part of our continuing interest in ascidian chemistry, we have examined cytotoxic extracts of the colonial ascidian, *Synoicum prunum* (Herdman, 1898). Three novel, weakly cytotoxic tetraphenolic bis-spiroketals, prunolides A (**1**), B (**3**), and C (**4**) and the known ascidian antibiotic, rubrolide A (**5**),[3] were isolated. This paper reports on the structures of the prunolides.



1 R=H, X=Br, Y=Br
2 R=Me, X=Br, Y=Br
3 R=H, X=Br, Y=H
4 R=H, X=H, Y=H



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Results and Discussion

S. prunum is a fist-sized yellow colonial ascidian which was collected off North Stradbroke Island, Queensland, Australia. The methanol extract of the freeze-dried ascidian inhibited the growth of cervical cancer cells (HeLa cells at 10 μg/mL). Repeated chromatography on sephadex LH20 led to the isolation of prunolide A (**1**) and prunolide C (**4**). A fraction containing impure prunolide B (**3**) was further purified by HPLC on C18. A highly colored late-eluting fraction from the sephadex LH20 was purified further by chromatography on diol-bonded silica, yielding rubrolide A (**5**).[3]

Prunolide A (**1**) was isolated as optically inactive yellow plates from DMSO. The high-resolution negative electrospray mass spectrum (HRESIMS) gave a $M - H^+$ ion at *m*/*z* 1204.3906, appropriate for a molecular formula, $C_{34}H_{14}{}^{79}Br_4{}^{81}Br_4O_9$ (Δ −5.0 mmu). Little structural information could be gained from inspection of the ^{1}H NMR spectrum (Table 1) since it consisted of only three signals, two aromatic singlets (δ 8.08 and 7.13), and one olefinic singlet (δ 7.03) integrating in a ratio of 2:2:1. The ^{13}C NMR spectrum was more informative since signals for 13 downfield carbons were observed. The intensity of four of these carbon resonances indicated that each could be assigned to coincident carbons, thus accounting for 17 carbons. It was clear that the molecule must have an axis of symmetry to account for the 34 carbons deduced from the mass spectral data. A DEPT experiment implied that the molecule contained 10 methine carbons, and thus the remaining four hydrogens could be assigned to exchangeable protons, although these were not observed in the ^{1}H NMR spectrum. A carbon signal at 167.3 ppm in the ^{13}C NMR spectrum and an IR absorption band at 1772 cm^{-1} were consistent with unsaturated ester carbonyls being present in the molecule. A further three downfield quaternary carbon resonances (157.4, 155.5, and 152.6 ppm) were consistent with two olefinic carbons β to carbonyls and four phenol groups, respectively.

Methylation of prunolide A with methyl iodide (reflux in acetone−K_2CO_3 for 2 h) afforded a tetramethyl derivative (**2**) as a colorless oil. The ^{1}H NMR spectrum of **2** contained two new aromatic methoxyl signals at δ 3.87 and 3.99. The chemical shift of the methoxyl carbons, 61.3 and 61.6 ppm, indicated that no protons were *ortho* to these aromatic methoxyl groups.

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10.1021/jo981881j CCC: $18.00
Published on Web 03/26/1999

Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz), HMBC, and ROESY NMR Data for Prunolide A (1) in DMSO-d_6

atom	^{13}C (mult, $^1J_{CH}$ Hz[a])	^{1}H (mult, J Hz)	$^{2,3}J_{CH}$ correlations	ROESY
1, 10	167.3 (s)	−	−	
2, 9	116.3 (d, 184)	7.03 (s, 2H)	167.3, 157.4, 115.3, 121.7	8.08, 7.13
3, 8	157.4 (s)	−	−	−
4, 7	115.3 (s)	−	−	−
5, 6	136.6 (s)	−	−	−
11, 29	121.7 (s)	−	−	−
12, 16, 30, 34	131.4 (d, 165)	8.08 (s, 4H)	112.4, 131.4, 152.6, 157.4	7.03, 7.13
13, 15, 31, 33	112.4 (s)	−	−	−
14, 32	152.6 (s)	−	−	−
17, 23	120.0 (s)	−	−	−
18, 22, 24, 28	132.0 (d, 165)	7.13 (s, 4H)	112.6, 132.0, 136.6, 155.5	7.03, 8.08
19, 21, 25, 27	112.6 (s)	−	−	−
20, 26	155.5 (s)	−	−	−

[a] Observed in a fully coupled HMQC spectrum.

Table 2. ^{1}H (600 MHz), ^{13}C (150 MHz), and HMBC NMR Data for Prunolide B (3) and Prunolide C (4) in DMSO-d_6

Prunolide B (**3**)				Prunolide C (**4**)		
^{13}C (mult[a])	^{1}H (mult, J Hz)	$^{2,3}J_{CH}$ correlations	atom	^{13}C (mult[a])	^{1}H (mult, J Hz)	$^{2,3}J_{CH}$ correlations
167.9 (s)	−	−	1, 10	168.7 (s)	−	
112.9 (d)	6.99 (s, 2H)	−	2, 9	114.0 (d)	6.77 (s, 2H)	168.7, 116.3
161.5 (s)	−	−	3, 8	161.1 (s)	−	−
115.5 (s)	−	−	4, 7	116.3 (s)	−	−
136.8 (s)	−	−	5, 6	136.7 (s)	−	−
120.8 (s)	−	−	11, 29	119.3 (s)	−	
132.0 (d)	8.11 (s, 4H)	112.9, 132.0, 158.1	12, 16, 30, 34	130.4 (d)	7.81 (d, 8.4 Hz, 4H)	130.4, 161.1, 161.4
112.9 (s)	−	−	13, 15, 31, 33	115.8 (d)	6.86 (d, 8.4 Hz, 4H)	119.3, 115.8, 161.4
158.1 (s)	−	−	14, 32	161.4 (s)	−	−
120.8 (s)	−	−	17, 23	120.0 (s)	−	−
132.2 (d)	7.14 (d, 2.4 Hz, 2H)	109.7, 128.4, 136.8, 155.3	18, 24	129.4 (d)	6.90 (d, 8.4 Hz, 2H)	129.4, 136.7, 158.3
109.7 (s)	−		19, 25	115.8 (d)	6.65 (d, 8.4 Hz, 2H)	115.8, 120.0, 158.3
155.3 (s)	−		20, 26	158.3 (s)	−	−
116.9 (d)	6.90 (d, 7.8 Hz, 2H)	109.7, 120.8, 155.3	21, 27	115.8 (d)	6.65 (d, 8.4 Hz, 2H)	115.8, 120.0, 158.3
128.4 (d)	6.85 (dd, 2.4, 7.8 Hz, 2H)	132.2, 136.8, 155.3	22, 28	129.4 (d)	6.90 (d, 8.4 Hz, 2H)	129.4, 136.7, 158.3

[a] Obtained from DEPT experiments.

A combination of gHMQC and gHMBC experiments (Table 1 for **1** and Experimental Section for **2**) indicated that prunolide A contained four 4-hydroxy-3,5-dibromophenyl groups, since correlations were observed between 8.08 and 112.4, 121.7, 131.4, 152.6 ppm and between 7.13 and 112.6, 120.0, 132.0, 155.5 ppm. A pair of 4-phenylfuranones was indicated from correlations between 7.03 and 167.3, 157.4, 115.3, 121.7 ppm, the carbon resonance at 115.3 ppm being assigned to the dioxygenated carbons C4 and C7. A correlation was observed between 7.13 and 136.6 ppm suggesting a 1,2-bis(4-hydroxy-3,5-dibromophenyl)ethylene was present in the molecule. Consistent with this assignment was a major fragment ion cluster centered at *m*/*z* 553.7382, assigned to 1,2-bis(4-methoxy-3,5-dibromophenyl)acetylene (calcd for $C_{16}H_{10}{}^{79}Br_2{}^{81}Br_2O_2$, Δ −0.9 mmu) which was observed in the electron impact mass spectrum of the tetramethoxyl derivative **2** of prunolide A. Assumption of bonds between C4−C5 and C6−C7 and oxygen bridges between C1 and C4, C4 and C7, and C7 and C10 led to structure **1**.

Eventually prunolide A crystallized from DMSO, and a single-crystal X-ray analysis confirmed the planar structure assigned by NMR analysis and indicated that the spiro-furanones were *trans* to each other.[4] The crystal data are as follows: $C_{34}H_{14}Br_8O_9.(C_2H_6SO)_2$, formula weight 1348.87, triclinic, space group *P*1 (no. 2), a = 13.62(1), b = 14.722 (7), c = 13.120 (6) Å, α = 93.48(4), β = 116.35(4)°, γ = 72.84(5), V = 2245(3) Å^3, Z = 2, D_{calc} = 1.995 g cm^{-3}, F_{000} = 1282.00 μ(Mo Kα) = 73.14 cm^{-1}.

Prunolide B (**3**) was obtained as an optically inactive yellow gum that gave a M − H$^+$ ion in the negative HRESIMS at *m*/*z* 1046.5768 corresponding to a molecular formula of $C_{34}H_{16}{}^{79}Br_3{}^{81}Br_3O_9$ (Δ −0.5 mmu). The elemental composition of **3** differed from that of **1** simply by the replacement of two bromine atoms by two hydrogen atoms, suggesting that prunolide B was a didebromo derivative of prunolide A. The ^{1}H NMR spectrum of **3** (Table 2) revealed that prunolide B (**3**) differed from prunolide A (**1**) only in the replacement of two 4-hydroxy-3,5-dibromophenyl residues attached to C5 and C6 in **1** with two 4-hydroxy-3-bromophenyl residues in **3**. COSY, gHMQC, gHMBC, and ^{13}C NMR experiments (Table 2) confirmed this assignment.

Prunolide C (**4**) was obtained as an optically inactive yellow gum that gave a M − H$^+$ ion at *m*/*z* 573.1196 ($C_{34}H_{21}O_9$; Δ 0.5 mmu) in the negative HRESIMS. COSY, gHMQC, gHMBC, ^{1}H NMR, and ^{13}C NMR experiments (Table 2) carried out on prunolide C (**4**) showed that it was the octadebromo derivative of prunolide A (**1**).

As prunolides A, B, and C each possesses a *C*2 axis of symmetry and should thus be chiral molecules, it is interesting to note that none of the compounds were optically active. This indicated that the three compounds were isolated as racemic mixtures.

The prunolides represent a structurally novel family of ascidian metabolites. The coisolation of rubrolide A (**5**) suggests that the prunolides probably arise from an

(4) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Center. The coordinates can be obtained, on request, from the CCDC Technical Editors, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax +44 1223 336033, or by email at deposit@ccdc.cam.ac.uk.

oxidative dimerization of a rubrolide precursor. The prunolides also bear some resemblance to the ascidian alkaloids polycitone A and polycitrins A and B,[5] and they probably share a common biogenesis.

Prunolide A (**1**) and prunolide C (**4**) were only weakly cytotoxic, inhibiting the growth of HeLa cells at a concentration of 25 μM and 15 μM, respectively. The major cytotoxic principles from the extract are still being investigated.

Experimental Section

General experimental details have been given in an earlier paper.[6]

X-ray Crystallography. Single crystals suitable for X-ray analysis were obtained as yellow thin plates from wet DMSO. Data were collected on a single crystal with dimensions 0.50 × 0.30 × 0.10 mm with a Rigaku rotating anode AFC7R diffractometer with graphite monochromated Mo Kα radiation λ 0.71069 Å using the $\omega-2\theta$ scan technique to $2\theta_{max} = 50°$ yielding 7900 independent reflections, 3690 with $I > 3\sigma(I)$ being considered observed. The intensities of three standards did not decrease significantly over the course of data collection. An empirical absorption correction was applied based on ϕ-scans with transmission factors ranging from 0.49 to 1.00. The structure was solved by direct methods, expanded using Fourier techniques and refined by full matrix least squares on $|F|$ (program TeXsan, 1992[7]). Non-hydrogen atoms were refined anisotropically. DMSO S atoms were disordered and modeled at 50% occupancy. $(x, y, z, U_{iso})_H$ were included and constrained at estimated values. Three hydroxyl protons were found by difference Fourier syntheses and included at constrained values. Weights derivative of $w = 1/[\sigma^2(F)]$ were employed. Conventional residuals R, R_w convergence were $R = 0.041$ and $R_w = 0.033$, gof = 2.23, $(\Delta/\sigma)_{max} = 0.00$, $\Delta\rho_{max} = 0.88$ e Å^{-3}.

Collection, Isolation, and Purification. *Synoicum prunum* was collected in May 1993 by SCUBA, −10 m, off North Stradbroke Island, Queensland, Australia. The methanol extract of the freeze-dried ground organism (18.5 g) was fractionated by repeated chromatography on Sephadex LH-20 (methanol), yielding prunolide C (**4**, 51.9 mg, 0.28%), impure prunolide B, and prunolide A (**1**, 100 mg, 0.53%), respectively. The fraction containing prunolide B was further purified by reverse phase HPLC, C18, elution with a solvent gradient from methanol/1% aqueous TFA (65:35) to methanol/1% aqueous TFA (80:20), yielding pure prunolide B (**3**, 1.6 mg, 0.009%). A later eluting fraction off the Sephadex column was chromatographed on diol-bonded silica (elution with dichloromethane and increasing percentages of methanol), yielding rubrolide A (**5**, 6 mg, 0.03%).

Prunolide A (1): obtained as yellow plates from DMSO, mp 212−214 °C. $[\alpha]^{25}_D = 0$ (*c*, 0.5, MeOH); UV (MeOH); λ_{max} 394.2 (ϵ 64500), 309.5 (79700) 210.5 (350200) nm; IR (film) ν_{max} 1772.2, 1541.9, 1380.7, 1306.8, 1209.1, 1158.5 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz) see Table 1; negative ESIMS, *m/z* (rel intensity %) 1212.7 (0.1), 1210.7 (4), 1208.7 (10), 1206.6 (16), 1204.3 (30), 1201.7 (20), 1199.6 (16), 1198.0 (10), 1196.1 (2); negative HRESIMS: M − H$^+$ 1204.3906 (calcd for $C_{34}H_{13}{}^{79}Br_4{}^{81}Br_4O_9$, Δ −5.0 mmu).

Prunolide B (3): obtained as a yellow gum. $[\alpha]^{25}_D = 0$ (*c*, 0.1, MeOH); UV (MeOH) λ_{max} 394.2 (ϵ 59600) 309.5 (70700) 215.0 (273500) nm; IR (film) ν_{max} 1771.0, 1481.2, 1209.2 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 2; ^{13}C NMR (DMSO-d_6, 150 MHz) see Table 2; negative LRESIMS, *m/z* (rel intensity %) 1052.8 (1), 1050.9 (10), 1048.8 (60), 1046.5 (100), 1044.1 (65), 1042.3 (50), 1040.2 (8); negative HRESIMS: M − H$^+$ 1046.5768 (calcd for $C_{34}H_{15}{}^{79}Br_3{}^{81}Br_3O_9$, Δ 0.3 mmu).

Prunolide C (4): obtained as a yellow gum. $[\alpha]^{25}_D = 0$ (*c*, 0.4, MeOH); $[\alpha]^{25}_D = 0$ (*c*, 0.3, MeOH); UV (MeOH) λ_{max} 314 (ϵ 37400), 210.3 (308500) nm; IR (film) ν_{max} 1758.7, 1605.7, 1513.9, 1291.0, 1214.4, 1150.2 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 2; ^{13}C NMR (DMSO-d_6, 150 MHz) see Table 2; LRESIMS, *m/z* (rel intensity %) 609 (M + Cl$^-$, 10), 573 (100). Negative HRESIMS: M − H$^+$ 573.1196 (calcd for $C_{34}H_{21}O_9$, Δ 0.5 mmu).

Tetramethyl Prunolide A (2). A stirred solution of prunolide A (5 mg) dissolved in dry acetone (10 mL), flame-dried, powdered potassium carbonate (500 mg), and methyl iodide (50 μL) was refluxed for 2 h. The reaction mixture was cooled to room temperature and filtered and the filtrate evaporated, yielding tetramethyl prunolide A (**2**) (5.1 mg, 100%) as a colorless gum. $[\alpha]^{25}_D = 0$ (*c*, 0.5, MeOH); UV (MeOH) λ_{max} 384.5 (ϵ 10300) 285.8 (99800), 210.3 (392000) nm; IR (film) ν_{max} 1786.5, 1529.8, 1474.4, 1300.5, 1205.8, 1070.7 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.87 s, 6H; 3.99, s, 6H; 6.40, s, 2H; 7.15, s, 4H; 7.89, s, 4H; ^{13}C NMR ($CDCl_3$, 150 MHz) 61.3 (20-OMe/26-OMe), 61.6 (14-OMe/32-OMe), 116.3 (C4/C7), 119.1 (C13/C15/C31/C33), 119.2 (C19/C21/C25/C27), 120.2 (C2/C9), 127.3 (C11/C29), 128.0, (C17/C23), 132.2 C12/C16/C30/C34), 132.4 (C18/C22/C24/C28), 137.9 (C5/C6), 156.1 (C20/C26), 157.5 (C3/C8), 157.7 (C14/C32), 167.0 (C1/C10); HMBC correlations: 3.87/156.2; 3.99/157.5; 6.40/116.3, 127.2, 157.8, 167.0; 7.15/119.2, 132.2, 137.9, 156.1; 7.89/119.1, 132.4, 157.5, 157.8. LREIMS, *m/z* (rel intensity %) 1269.4 (0.1), 1267.4 (2) 1265.5 (4), 1263.4 (8) 1261.4 (10), 1259.4 (8), 1257.4 (4), 1255.4 (1.5), 1253.4 (0.2), 949.5 (5), 947.5 (10), 945.5 (25), 943.5 (30), 941.5 (24), 939.5 (9), 937.5 (2), 625.7 (5), 553 (5), 538 (7), 291.8 (55), 289.8 (100), 287.8 (54), 276.0 (15), 274.0 (24), 272.0 (12). HREIMS: M$^+$ 1259.4719 (calcd for $C_{38}H_{22}{}^{79}Br_5{}^{81}Br_3O_9$, Δ 5.3 mmu), (M$^+$ − $C_{10}H_6Br_2O_2$) 943.6009 (calcd for $C_{28}H_{16}{}^{79}Br_3{}^{81}Br_3O_7$, Δ −7.3 mmu), (M$^+$ − $C_{22}H_{12}Br_4O_6$) 553.7382 (calcd for $C_{16}H_{10}{}^{79}Br_2{}^{81}Br_2O_7$, Δ −0.9 mmu), (M$^+$ − $C_{29}H_{16}Br_6O_8$) 289.8760 (calcd for $C_9H_6{}^{79}Br{}^{81}BrO$, Δ 0.5 mmu).

Acknowledgment. Financial support is acknowledged from the Australian Research Council. We thank Mr. John Kennedy from the Queensland Museum for identification of the ascidian, and Dr. Dave Bourne and Dr. John MacLeod, Research School of Chemistry at the Australian National University, for EI mass spectrometric analysis. We thank Mr. Rick Willis, Australian Institute of Marine Science, for high resolution electrospray mass spectrometric analysis. We are indebted to Dr. Greg Fechner and Ms. Rama Addepalli from the Queensland Pharmaceutical Research Institute, Griffith University, for cytotoxicity screening.

Supporting Information Available: ^{1}H NMR spectra for prunolides A−C (**1**, **3**, and **4**) and tetramethylprunolide A (**2**). An ORTEP diagram of prunolide A and tables of crystal data including bond lengths and angles, atomic coordinates, and anisotropic thermal parameters for prunolide A. This material is available free of charge via the Internet at http://pubs.acs.org.

JO981881J

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