Tetillapyrone and Nortetillapyrone, Two Unusual Hydroxypyran-2-ones from the Marine Sponge *Tetilla japonica*

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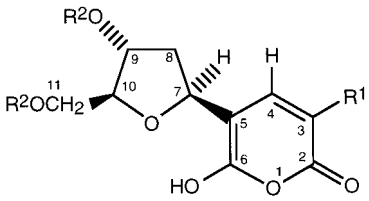
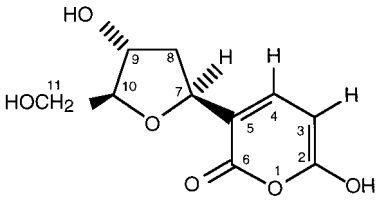
Extraction of the marine sponge *Tetilla japonica* from the Bay of Thailand furnished tetillapyrone and nortetillapyrone, two unusual tetrahydrofurylhydroxypyran-2-ones, whose structures were established by NMR spectrometry and an X-ray analysis of tetillapyrone.

There is little chemical information on sponges of the family Tetillidae, order Spirophorida, class Demospongia. Previous workers have described lipids from *Cynachyrella alloclada*,[1,2] and 3*β*-*O*-methylsecosteroids have been isolated from *Jereicopsis graphidiaphora*,[3] while Fusetani and co-workers[4] reported isolation in very small amount of the potent cytotoxic macrolide cynachyrolide A from a marine sponge of the genus *Cynachyra*, which apparently is identical with spongistatin 4, one of a group of equally potent macrolides isolated from marine sponges of the genera *Spongia*, *Spirastrella*, and *Hyrtios*.[5] We have now studied a collection of *Tetilla japonica* Thiele from the Gulf of Thailand. In addition to 24-methylenecholest-5-en-3*β*-ol, two unusual 5-tetrahydrofurylhydroxypyran-2-ones, **1a** and **2**, which we have named tetillapyrone and nortetillapyrone, were isolated.



1a $R^1 = CH_3$, $R^2 = H$
b $R^1 = CH_3$, $R^2 = Ac$
c R^1, $R^2 = H$



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Results and Discussion

Structure and stereochemistry of the left half common to both substances, a 2-substituted 4-hydroxy-5-hydroxymethyltetrahydrofuran, were easily deduced from the ^1H and ^{13}C NMR spectral data listed in Tables 1 and 2. In both instances COSY and NOESY correlations established that a low-field proton (H-7), resonating near *δ* 6.17 and alpha to the oxygen of the tetrahydrofuran ring, was adjacent to a methylene group, trans to a proton (H-9) deshielded by a hydroxyl and cis to a proton (H-10) on carbon carrying a $-CH_2OH$ group. Formation of a diacetate, **1b**, whose ^1H and ^{13}C NMR spectra (Table 1) reflected the expected changes confirmed the assignments, although the unusual chemical shift (*δ* 6.17) of the proton at C-2 of the tetrahydrofuran ring demanded attention.

On the other hand structure elucidation of the right halves of both molecules presented difficulties. In the case of **1a** the ^1H NMR spectrum indicated the presence of a vinylic hydrogen at *δ* 7.69 (H-4 in the final formula, C-4 at *δ* 136.22), apparently at the *β*-position of a conjugated ketone or lactone and allylically coupled to a vinyl methyl at *δ* 1.76. The latter was absent in the case of the nor-derivative and replaced by another vinylic hydrogen at *δ* 5.63. By HMBC the vinylic hydrogen of **1a** at *δ* 7.69 was attached to a carbon atom two bonds removed from a second carbon represented by a singlet at *δ* 150.41, apparently also vinylic or aromatic and possibly attached to a third hydroxyl group whose signal appeared at *δ* 11.28. The third hydroxyl group was not affected by conversion of **1a** to its diacetate and was not enolic, as shown by the absence of a positive $FeCl_3$ test and by the lack of change in the UV absorption on addition of base.

The 10 carbons, 14 hydrogens, and four oxygens—three belonging to the three hydroxyls and one to the tetrahydrofuran ring—deduced from the ^1H and ^{13}C NMR evidence presented in the previous paragraphs accounted for only 198 of the 242 mass units in the mass spectrum of tetillapyrone, leaving apparently one carbon and two oxygen atoms unaccounted for. Since the substance furnished suitable crystals, an X-ray analysis was therefore undertaken. The surprising result is displayed in Figure 1, which shows that the substance is a 5-tetrahydrofuryl-6-hydroxypyran-2-one, **1a**, which in the solid state is essentially ionic, i.e., in the form of an enolate. In the crystal there was no evidence for a hydrogen atom on O-2

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Table 1. ^{1}H and ^{13}C NMR Data of **1a** and **1b**[a]

	1a					1b		
position	δH mult (Hz)[b]	δC mult[c]	NOESY	COSY	HMBC	δH mult[b]	δC mult[c]	HMBC
2		163.84 s					163.61 s	
3		109.45 ?					109.45 ?	
4	7.69 d (1)	136.22 d	H-7,8,9,Me, 11-OH	Me	C-2,6,Me-C	7.50 d (1)	135.77 d	C-2
5		109.45 s					109.93 s	
6		150.54 s					150.41 s	
7	6.16 t (6.8)	83.81 d	H-4,8,10	H-8a	C-4,6	6.17 dd (8,6.3)	83.95 d	C-4,6
8a	2.09 m	39.47 t		H-7,8b,9	C-7	2.42 m	35.44 t	
8b	2.05 m			H-7,8a,9	C-7	2.26 m		
9	4.73 dq (4,3.5)	70.51 d	H-8,10,11,9-OH, 11-OH	H-8a,b,10, 9-OH		5.18 m	73.90 d	C-7
10	3.75 q (3.5)	87.83 d	H-7,9-OH	H-9,11a,b		4.14 m	81.00 d	
11a	3.59 ddd (12,5.1,3.5	61.40 t	H-9,10,11-OH	H-10,11b, 11-OH		4.24 d (5)		
11b	3.54 ddd 12,5.1,3.5)					4.24 d (5)	63.63 t	C-9,10,Ac
CH_3	1.76 brs	12.33 q	H-4	H-4	C-2,4,5	1.79 brs	12.13 q	C-2,4,5
6-OH	11.28 brs				C-5	11.39 s		C-4
9-OH	5.24 d (4.2)		H-8,9,10	H-9	C-8,9,10			
11-OH	5.04 t (5.1)		H-9,11	H-11a,b				
Ac						2.08 s, 2.06 s	170.15, 170.03, 20.75, 20.58	

[a] Spectra recorded in DMSO-d_6. [b] *J* values in parentheses. [c] Multiplicities deduced by DEPT.

Table 2. ^{1}H and ^{13}C NMR Data of **1c**[a]

position	δH mult (Hz)[b]	C mult[c]	COSY	HMBC
2		163.18 s		
3	5.63 d (8.1)	101.79 d	H-4	C-2,4
4	7.84 d (8.1)	140.56 d	H-3	C-2,6,7
5		?		
6		150.48 s		
7	6.15 t (6.6)	84.14 s	H-8a,b	C-4,6
8a	2.11 m	39.70 t	H-7,9	C-7,9
8b	2.04m		H-7,9	C-7,9
9	4.22 dq (4,3.5)	70.46 d	H-8a,b,10,9-OH	
10	3.77 q (3.5)	87.43 d	H-9,11a,b	
11a	3.55 m	61.31 t	H-10,11-OH	
11b	3.51 m		H-10,11-OH	
2-OH	11.30 brs		H-3	
9-OH	5.26 d (4.1)		H-9	C-9,10
11-OH	5.03 t (5.1)		H-11a,b	C-10

[a] Spectra recorded in DMSO. [b] *J* values in parentheses. [c] Multiplicities deduced by DEPT.

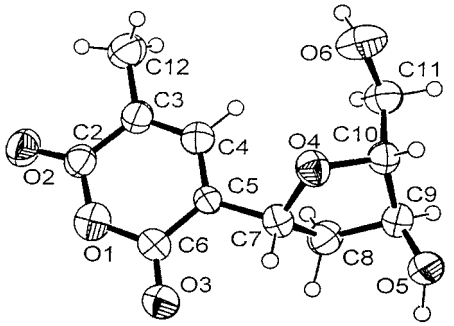

Figure 1. ORTEP diagram (50% probability ellipsoids) showing crystallographic numbering scheme and solid state conformation of tetillapyrone (**1a**).

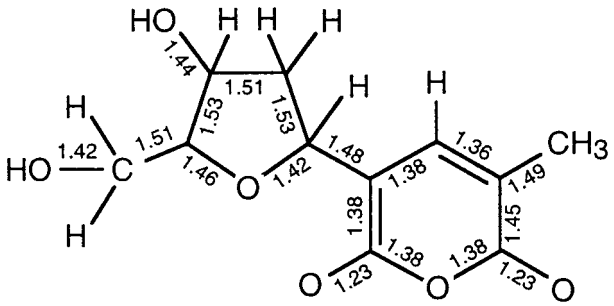

Figure 2. Bond distances in **1a**.

or O-3, the molecular mass by X-ray diffraction corresponding to *m*/*z* 241 rather than to the *m*/*z* 242 required by the mass spectrum. Bond lengths in the crystal are shown in Figure 2; the C-2−O-2 and C-6−O-3 bond lengths correspond to those of carbonyl groups, the C-2−O-1 and C-6−O-1 bonds are also equal in length and correspond to C−O single bonds. Three of the ring C−C bonds are approximately equal in length to those in benzene, while the C-2−C-3 bond is longer but still shorter than a C−C single bond.

In solution tetrahydropyrone can, however, be represented as **1a**. The HMBC data of Table 1 further suggest that the chemical shifts of C-3 and C-5 fortuitously coincide since the methyl group on C-3 of the pyrone ring apparently correlates with C-5 as well as with C-2 and C-4 and since the enolic hydroxyl also correlates with C-5.

In the case of nortetillapyrone (Table 2) the C-5 signal also appears to be superposed on one of the other pyrone ring carbon signals. A COSY correlation between H-3 and the low-field, presumably enolic, hydroxyl proton on the pyrone ring indicates that in this instance the hydroxyl is on C-2; that is, the formula of nortetillapyrone in solution seems to be **2** rather than **1c**.[9] The downfield shifts of H-7 in both compounds can be attributed to the ring current of the essentially aromatic 5-alkyl-6-hydroxypyran-2-one system. The two substances can be viewed as α-substituted glutaconic anhydrides, which like synthetic *β*-substituted glutaconic anhydrides[10−12] would be in equilibrium with the enol or 6-hydroxypyran-2-one form. In the present instance the equilibrium in solution is apparently entirely on the side of the enol.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker AMC instrument operating at 300.13 and 75.47 MHz, respectively. EI mass spectra were measured on a Hitachi Perkin-Elmer RMU-6M instrument. HRMS samples were run using + FAB ionization with Xe gas at 6 kV on a Kratus Concept III, 2 sector mass spectrometer. The accelerating voltage was 8 kV. UV spectra were run on a Cary 1E UV−visible spectro-

photometer. Rotations were run on a Polarotronic Universal Schmidt and Haensch polarimeter. Si gel for column chromatography was Si gel 60 (0.2−0.5 mm) Merck, and for analytical and preparative TLC Si gel G-60 GF 254 Merck.

Animal Material. *Tetilla japonica*, family Tetillidae, order Spirophorida, class Demospongiae, was collected by S. Puchakarn using scuba in January 1999 at Captain Yuth Beach, Chonburi, Thailand, at 3−5 m depth and immediately frozen. The collection was identified by Prof. Rob van Soest, Department of Coelenterates and Porifera, Zoological Museum, University of Amsterdam. A sample (voucher number BIMS-I951)was deposited at the Reference Collection Museum of the Marine Science Institute, Burapha University, Chonburi, 20131, Thailand.

Extraction and Isolation. The sample (3 kg wet wt) was thawed, thoroughly homogenized with EtOH, and extracted with EtOH (3 × 1000 mL). The extract was filtered and concentrated at reduced pressure to 800 mL, which resulted in removal of most of the EtOH, and extracted with EtOAc (3 × 500 mL). The EtOAc extract was concentrated at reduced pressure to give a residue (23.8 g), which was chromatographed over Si gel (480 g) and eluted with petroleum ether−$CHCl_3$ and $CHCl_3$−MeOH, 200 mL fractions being collected as follows: 1−42 (petrol−$CHCl_3$, 3:2), 43−61 (petrol−$CHCl_3$, 1:1), 62−100 (petrol−$CHCl_3$, 3:7), 109−129 (petrol−$CHCl_3$, 1:9), 130−162 ($CHCl_3$−MeOH), 163−188 ($CHCl_3$−MeOH, 19:1), 189−215 ($CHCl_3$−MeOH, 9:1), 216−230 ($CHCl_3$−MeOH, 4:1). Fractions 12−16 (2.9 g) were recrystallized from petroleum ether to give 251 mg of 24-methylenecholest-5-en-3β-ol identified by MS and ^{1}H NMR spectrometry. Fractions 189−200 (189 mg) were purified by TLC (Si gel, $CHCl_3$−Me_2O−HCO_2H, 35:65:1) to give tetillapyrone (69 mg) and nortetillapyrone (19 mg). Fractions 201−215 (145) were purified by TLC (Si gel, $CHCl_3$−Me_2O−HCO_2H, 35:65:1) to give additional amounts of tetillapyrone (19 mg) and nortetillapyrone (26 mg).

Tetillapyrone, (7*R,9*S**,10*R**)-3-methyl-5-[4-hydroxy-5-hydroxymethyltetrahydrofuryl]-6-hydroxypyran-2-one (1a):** colorless needles ($CHCl_3$−MeOH); mp 191−192 °C; $[\alpha]_D^{20}$ +17.6° (*c*, acetone), UV (MeOH) λ_{max} (log ϵ) 20.7 (3.86), 267 (3.85); ^{1}H and ^{13}C NMR spectra in Tables 1 and 2; EIMS *m*/*z* 242 (M^+, 55), 211 (10), 206 (15), 153 (50), 126 (35), 117 (100) 110 (18), 99 (28), 73 (42); however it was not possible to obtain peaks for accurate masses by high-resolution FAB or EI/CI, although at low resolution such a mass spectrum exhibited a strong peak at *m*/*z* 265 (M^+ + Na).

Acetylation of **1a** in the usual manner afforded a noncrystalline diacetate **1b**; EI-MS *m*/*z* 326 (M^+, 18), 201 (100), 193 (20), 140 (20), 126 (50), 110 (23), 98 (25); ^{1}H and ^{13}C NMR in Table 1.

Nortetillapyronpyrone, (7*R,9*S**,10*R**)-3-[4-hydroxy-5-hydroxymethyltetrahydrofuryl]-6-hydroxypyran-2-one (2):** a gum; $[\alpha]_D^{20}$ +8.6° (*c*, 0.35, acetone); UV λ_{max}^{MeOH} (log ϵ) 205 (3.50), 262 (3.36); ^{1}H and ^{13}C NMR spectra in Table 2; EI MS *m*/*z* 228 (M^+, 27), 210 (13), 198 (13), 192 (89), 168 (14), 139 (45), 126 (20), 117 (100), 112 (19), 73 (32); attempts at HRMS by FAB or EI/CI met with the same difficulties encountered in the case of **1a**.

X-ray Analysis of 1a. Crystals suitable for X-ray diffraction were obtained by slow evaporation of a saturated acetone solution and belonged to space group $P2_12_12_1$, cell dimensions a = 4.863(2) Å, b = 13.949(6) Å, c = 16.366(9) (uncertainties in parentheses), Z = 4, calcd density 1.44 g/cm^3. X-ray diffraction studies were performed at room temperature with a Stoe IPOS image plate equipped with Mo Kα radiation; chemical formula weight from the X-ray analysis of $C_{11}H_{13}O_6$ (241.72). A total of 8695 reflections were measured, of which 2115 were independent and 1685 were observed ($I > 2\sigma(I)$). The structure was solved using SHELX 97[13] and refined with SHELXL 97.[14] Non-hydrogen atoms were refined anisotropically; the refinement converged to $R(F)$ = 7.95% and $wR(F^2)$ = 16.24%. All hydrogen atoms except those of the ring methyl group were found in the difference Fourier map; therefore the hydrogen atoms around this methyl group were calculated assuming a tetrahedral environment for the carbon atom and refined using the riding model.

The ring O1−C2−C3−C4−C5−C6 defines a plane with rms deviation of 0.0038 Å. The second ring is not planar, and while C10−O4−C7−C8 lies on a plane with rms deviation of 0.0305 Å, C9 deviates 0.576(6) Å from this plane. In the crystal there are two intermolecular hydrogen bonds [O5−H···O2′ ($-x$, y − 0.5, $-z$ + 1.5), 2.736(4) Å, and O6−H···O6′ (x − 0.5, $-y$ + 1.5, $-z$ + 1.5, $-z$ + 1), 2.793(3) Å]. A perspective view of the molecule was obtained with ORTEP[15] and is shown in Figure 1.

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