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Polyclinal, a new sulfated polyhydroxy benzaldehyde from the marine ascidian *Polyclinum planum*

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Summary. A new sulfated polyhydroxy benzaldehyde has been isolated from extracts of the temperate colonial ascidian *Polyclinum planum.* The structure of the new metabolite was solved by an X-ray crystallographic study. The highest concentration of this metabolite was found in the zooid-rich outer layers of this ascidian suggesting that it may represent a potential chemical defense against predators.
Key words. Polyclinum planum; ascidian; sulfonated benzaldehyde.

The soft bodies of many ascidians and their tendency to grow on exposed substrates would appear to make them easily accessible to a variety of potential predators[3]. Ascidians are rich sources of bioactive secondary metabolites that show potent antimicrobial, cytotoxic and antiviral activities[4], but only one study using ecologically relevant field assays has demonstrated an ichthyodeterrent function of secondary metabolites from an ascidian[5]. While the majority of chemically-rich ascidians have been collected from predator-rich water of the Caribbean and Indo-Pacific, ascidians from temperate marine habitats have also been identified as important sources of novel bioactive secondary metabolites[6].

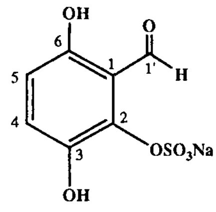
In a survey of physically vulnerable ascidians in the temperate Pacific waters off La Jolla, California, the colonial ascidian *Polyclinum planum* was found to contain significant quantities of a UV-active secondary metabolite. In this paper we describe the isolation and structural elucidation of a sulfated trihydroxy-benzaldehyde derivative from *P. planum* and examine its distribution within the colonies.

Materials and methods

The colonial ascidian *Polyclinum planum* (Polyclinidae, Aplousobranchia) was a common member of the benthic invertebrate fauna at the Fish and Game Reef near La Jolla, California, throughout 1987. Collections of *P. planum* were frozen and lyophilized. The lyophilized ascidian was then exhaustively extracted with acetone. The reduced acetone extract was partitioned between hexane and methanol, and the methanol soluble material was fractionated by silica gel vacuum flash chromatography. Gel-filtration of one of the fractions with Sephadex LH-20, using methanol as the eluting solvent, yielded polyclinal (**1**) as the only secondary metabolite at 0.13% dry weight of the ascidian.

Structure of polyclinal (**1**). The ^{1}H NMR spectrum of **1** exhibited only three proton resonances, while in the ^{13}C NMR spectrum one carbonyl and six benzenoid aromatic resonances were observed[7]. An aromatic aldehyde was indicated by the proton singlet at δ 10.26 and the methine ^{13}C resonance at δ 196.8. An IR absorption at 1670 cm^{-1} confirmed the presence of this aromatic aldehyde. The ^{1}H NMR spectrum of **1** showed *ortho* aromatic protons δ 7.27 (1H, d, $J = 6.8$ Hz) and 6.68 (1H, d, $J = 6.8$ Hz) which suggested a 1,2,3,4 substitution pattern for this benzaldehyde derivative. The two ^{13}C NMR bands at δ 143.5 and 142.1 illustrated the presence of two free phenolic hydroxyl groups in **1** and the UV spectrum in methanol [368 (ε 4200), 264 (9300), 232 nm (12 000)] was appropriate for a polyhydroxylated benzaldehyde[8]. The presence of dimers and trimers of **1** was indicated by the observation of high mass ions at *m/z* 757, 533, 511 and 489 in the FAB mass spectrum but no plausible molecular ion for **1** could be determined from these data.

Acetylation of **1** with acetic anhydride in pyridine afforded the penta-acetate **2** as the sole reaction product. The presence of five acetate groups[9] indicated that **2** was the aldehyde-hydrate diacetate derivative. The new C-1' methine proton was observed at δ 7.98 (s). The subsequent loss of ketene from the highest mass ion in the LRDEI mass spectrum of **2** (at *m/z* 323) produced fragments at *m/z* 280, 238, 196 and 154.



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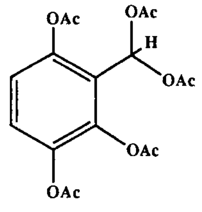

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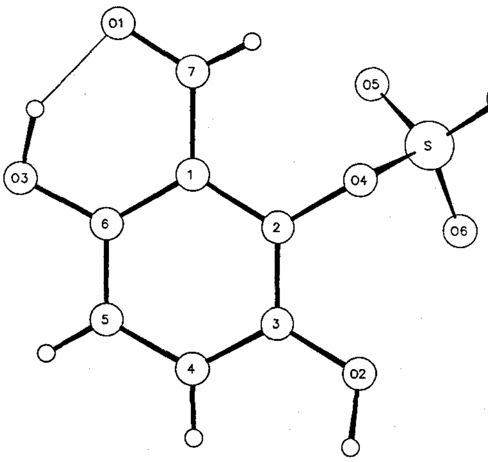
No structures could be confidently assigned to either **1** or **2** based on the available spectral and chemical data, hence the structure of polyclinal (**1**) was determined through an X-ray crystallographic experiment[10]. The X-ray structure (fig.) of polyclinal confirmed the existence of the aromatic aldehyde and phenolic groups, but the presence of the C-2 sodium sulfate group was unexpected. On the basis of the X-ray structure of **1**, the structure of the penta-acetate derivative was thus established as **2**. Among polyhydroxylated benzaldehydes isolated from marine sources, only the siphonodictyals[11–13], including



An X-ray perspective drawing for polyclinal (**1**).

the sulfated metabolite siphonodictyal D isolated from the burrowing sponge *Siphonodictyon coralliphagum*, possess an analogous substitution pattern of phenolic hydroxyl groups as polyclinal (**1**).

Subsequent collections of *P. planum* were used to investigate the distribution of polyclinal within three distinct regions of the ascidian colonies. The colonies were dissected into the stalk, the pulpy inner mesenchyme and the zooid-rich surface layer of the colonies. The concentrations of polyclinal in these different colony parts, based on wet weights, were determined to be 5.8×10^{-5} g/g, 7.8×10^{-4} g/g and 2.5×10^{-3} g/g in the stolons, the cortex and the zooid rich outer layers of the colonies, respectively. The higher concentration of polyclinal in the zooid-rich surface layer of the colonies suggests that polyclinal may function as a chemical defense against predators which would be consistent with previous observations on the distribution of predator deterrent gorgonian secondary metabolites in the outer more accessible portions of the colonies[14]. Due to the instability of polyclinal in the agar-squid preparations used in our feeding preference assays, we were unable to perform ecologically relevant bioassays to investigate this metabolite's potential ichthyodeterrent properties.

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7 Polyclinal (**1**) ^{1}H NMR (CD$_3$OD, 360 MHz) 10.26 (1H, s, H-1'), 7.27 (1H, d, J = 6.8 Hz, H-4), 6.68 (1H, d. J = 6.8 Hz, H-5). ^{13}C NMR (CD$_3$OD, 50 MHz) 196.8 (C-1'), 157.0 (C-6), 143.5 (C-3), 142.1 (C-2), 128.6 (C-4), 116.4 (C-1) 115.6 (C-5) IR (nujol) 3550-3350, 1670, 1640, 1630, 1585, 1300, 1220, 1180, 1075, 1038, 950 cm^{-1}. Negative LR-FABMS obsd. *m/z* 767, 533, 511, 489, 255 and 233.
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9 Derivative **2**. ^{1}H NMR (CD$_3$OD, 360 MHz) 7.98 (1H, s, H-1'), 7.31 (1H, d, J = 6.8 Hz, H-4), 7.05 (1H, d, J = 6.8 Hz, H-5), 2.37 (6H, s, -OAc), 2.26 (3H, s, -OAc), 2.03 (6H, s, -OAc). LRDEIMS obsd. *m/z* 323, 280, 238, 196, and 154.
10 Polyclinal crystalized in the common monoclinic space group P2$_1$/c with a = 8.588 (2), b = 10.405 (1), c = 10.304 (2) A, and β = 101.30 (1)°. The structure was solved routinely, and the conventional crystallographic residual for the 1128 (93%) observed ($|F_0| > 3\sigma(F_0)$) reflections is 0.047. Archival X-ray crystallographic data have been deposited with and can be ordered from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. Please give a complete literature citation when ordering.
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New tambjamine class alkaloids from the marine ascidian *Atapozoa* sp. and its nudibranch predators. Origin of the tambjamines in *Atapozoa*

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Summary. Two new tambjamine class alkaloids, possessing ichthyodeterrent properties, have been isolated from the organic extracts of the marine ascidian *Atapozoa* sp. and its nudibranch predators. The structure of the new metabolites were elucidated through interpretation of their physical and spectral data and by comparison with spectral data for related compounds. Microscopic examination of *Atapozoa* considering the yellow color of the tambjamines suggested that *Atapozoa* is capable of the de novo biosynthesis of these metabolites.
Key words. Atapozoa; ascidian; chemical defense; tambjamine class alkaloids; origin of secondary metabolites.