

## Isolation and X-ray Structure of Deoxycholic Acid from the Sponge *Ircinia* sp.

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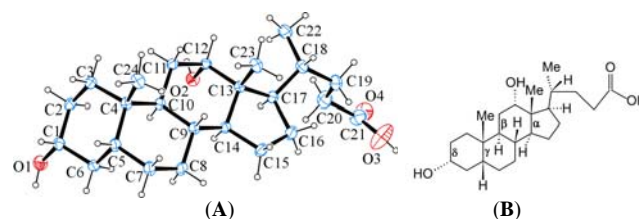
Ethyl acetate extract of the sponge *Ircinia* sp., collected by SCUBA divers off the coast of southern India, contained a cholest-based sterol of insignificant antimicrobial activity, with one carboxylic acid side chain (3 $\alpha$ , 7 $\beta$ -dihydroxy-5 $\beta$ -cholan-24-oic acid, DCA). The structure of the compound was determined by spectroscopic data and single crystal X-ray diffraction studies. DCA crystallizes in the tetragonal space group P4<sub>1</sub>2<sub>1</sub>2, chirality documented by solution optical rotation  $[\alpha]_D = +67.4^\circ$  (c 0.16, CHCl<sub>3</sub>).

**Keywords:** Sterol, *Ircinia* sp., deoxycholic acid, marine sponges, spectroscopy, crystallography.

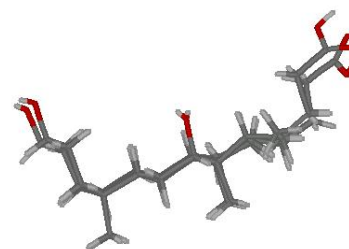
Marine life is ripe of organisms that have specialized to survive by developing strategies which involve production of certain secondary metabolites that are not common in terrestrial life. In view of this, we are interested to distract those compounds from marine life forms which could be of medicinal importance, see also [1]. Here, we present the spectroscopic characterization of a compound from the ethyl acetate extract of the sponge *Ircinia* sp.

The compound crystallized in space group P4<sub>1</sub>2<sub>1</sub>2 of the tetragonal crystal system revealing two independent molecules of 3 $\alpha$ ,12 $\beta$ -dihydroxy-5 $\beta$ -cholan-24-oic acid (deoxycholic acid, DCA) and one water molecule in the asymmetric unit. The unit cell contains a total of 8 molecules. Relative chiral centers for both the independent molecules were assigned as (1R\*, 4S\*, 5R\*, 9R\*, 10S\*, 12S\*, 13R\*, 14S\*, 17R\*, 18R\*), (Figure 1). The absolute structure could not be obtained with Mo-radiation. Instead we document its chirality by solution optical rotation  $[\alpha]_D = +67.4^\circ$  (c 0.16, CHCl<sub>3</sub>) to allow for correlating any further investigation to this naturally occurring compound.

DCA is composed of four rings depicted as  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  in Figure 1B. Rings  $\alpha$ ,  $\beta$ , and  $\gamma$  are considered rigid, whereas ring  $\delta$  can take *cis*- or *trans* conformation relative to the side chain structure. In both independent molecules, the configuration of ring  $\delta$  is *cis*. Differences between the independent molecules appear in a slightly more expressed 'bend' in molecule I (see Table 2) and rotation of the carboxyl groups between the molecules (Figure 2). The bond lengths and bond angles in the two DCA molecules are comparable and the values are within the expected ranges [2]. The C-O bond distances are 1.442(4)Å and 1.432(3)Å for the ring hydroxyl groups which is slightly



**Figure 1** (A): ORTEP diagram of one of two similar deoxycholic acid molecules at room-temperature with thermal ellipsoid probabilities at the 20% level. (B): 3 $\alpha$ ,12 $\beta$ -dihydroxy-5 $\beta$ -cholan-24-oic acid molecule.  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  depict different rings;



**Figure 2.** Overlay of the two symmetry-independent molecules of DCA which shows different conformations of the carboxylic groups.

longer than the C-O bond distance of side chain carboxylic hydroxy (1.327(6)) Å. The mean values of C-C-C bond angles are 103(3) $^\circ$  and 110(9) $^\circ$  for the five member and six member polycyclic skeletons respectively, while the C-C-C bond angle for the side chain is 113(6) $^\circ$ . Several other structures of DCA have previously been established by X-ray crystallography showing a large variety of motifs [3a,3b]. Most of the structures reported belong to monoclinic, hexagonal and orthorhombic crystal systems [3c]. Specifically, a monoclinic structure was reported for DCA co-crystallizing with *o*-xylene [3d] at a ratio of 2:1 and an orthorhombic structure is known for DCA with a salicylic complex [3e].

**Table 1.** Crystal structure determination and refinement of DCA.

Empirical formula	C <sub>48</sub> H <sub>82</sub> O <sub>9</sub>
Empirical formula weight	803.14
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Tetragonal
Space group	P 4 <sub>1</sub> 2 <sub>1</sub> 2
Unit cell dimensions	a = 14.1235(2) Å, α = 90° b = 14.1235(2) Å, β = 90° c = 47.2827(8) Å, γ = 90°
Volume	9431.6(2) Å <sup>3</sup>
Z	8
Density (calculated)	1.131 Mg/m <sup>3</sup>
Absorption coefficient	0.076 mm <sup>-1</sup>
F(000)	3536
Crystal size	0.35 x 0.30 x 0.30 mm <sup>3</sup>
Theta range for data collection (°)	1.50 to 30.60
Index ranges	-20 ≤ h ≤ 19, -18 ≤ k ≤ 18, -66 ≤ l ≤ 65
Reflections collected	52326
Independent reflections	8159 [R(int) = 0.0364]
Completeness to theta	25.00°, 99.8 %
Max. and min. transmission	0.9776 and 0.9739
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	8159 / 0 / 526
Goodness-of-fit on F <sup>2</sup>	1.021
Final R indices [I > 2σ(I)]	R <sub>1</sub> = 0.0661, wR <sub>2</sub> = 0.1837
R indices (all data)	R <sub>1</sub> = 0.1108, wR <sub>2</sub> = 0.2159
Largest diff. peak and hole	0.541 and -0.270 e.Å <sup>-3</sup>

**Table 2.** Selected bond lengths (Å) and angles (°) for DCA

Molecule I		Molecule II	
<i>Bond lengths</i>			
C1-O1	1.442(4)	C25-O5	1.424(5)
C12-O2	1.432(3)	C36-O6	1.427(4)
C21-O3	1.327(6)	C45-O7	1.258(6)
C21-O4	1.188(5)	C45-O8	1.158(7)
C19-C20	1.470(5)	C43-C44	1.508(7)
C16-C17	1.546(5)	C40-C41	1.555(6)
C13-C14	1.542(4)	C37-C38	1.536(5)
C4-C5	1.544(4)	C28-C29	1.543(5)
<i>Bond angles</i>			
C18-C19-C20	114.6(3)	C42-C43-C44	113.5(5)
C17-C18-C19	112.7(3)	C41-C42-C43	112.3(4)
C19-C20-C21	113.5(4)	C43-C44-C45	113.5(5)
C2-C1-C6	109.9(3)	C26-C25-C30	110.4(4)
C15-C16-C17	107.3(3)	C39-C40-C41	107.4(4)

Antimicrobial screening by disk diffusion method showed that the compound presented here is of insignificant antimicrobial activity.

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## Experimental

**Animal Material:** Specimens of *Ircinia* sp. were collected by hand by divers equipped with SCUBA off the coast of southern India at a depth of 2-10 m. Reference voucher have been deposited at the National Institute of Oceanography, Goa, under the voucher no. NIO-859.

**Extraction and Isolation:** Dried and minced specimens of the sponge (dried using lyophilizer) were dissolved with petroleum ether, ethyl acetate and *n*-butanol to give three different extracts. The ethyl acetate extract was subjected to flash column chromatography over silica gel mesh 230-400. Ethyl acetate: Petroleum ether (v/v) solvent system was used as eluant with increasing polarity of ethyl acetate (0:100 to 100:0) and finally 200 ml of methanol to remove polar compounds from the column, leading to collection of 32 fractions. Fractions 15-22 were combined and further separated on silica gel column using increasing amount of methanol in chloroform yielding a pale white solid which was recrystallized to give deoxycholic acid (38 mg).

**X-ray Crystallographic studies:** Crystals of suitable quality for X-ray analysis were grown from CHCl<sub>3</sub> and methanol (2:1). X-ray structural data was obtained using a Bruker Apex II single crystal X-ray diffractometer employing Mo-Kα radiation (λ = 0.71073 Å). Crystal-to-detector distance was 40 mm and exposure time was 120 seconds per frame for all sets collected at steps of 0.5°. Data collection was 99.8% complete to 25° in θ. Data was integrated and scaled using SAINT, SADABS, within the APEX2 software packages [4a] The structure was solved by direct methods (SIR [4b]) and refined by full matrix least squares based on F<sup>2</sup> using SHELXL-97 [4c] and WinGX [4d].

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