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A Novel Group of Polyhydroxycholanic Acid Derivatives from the Deep Water Starfish *Styracaster caroli*

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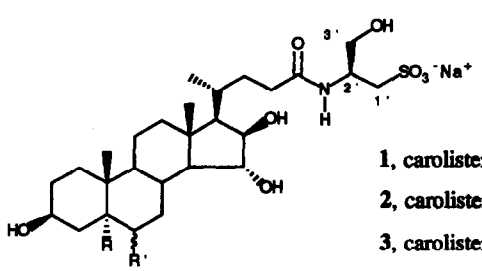
Key Words: marine natural products; starfish; polyhydroxysteroids; polyhydroxycholanic acid; cysteinolic acid.

Abstract: *Three novel polyhydroxysteroid constituents have been isolated from the starfish Styracaster caroli collected at a depth of 2000 m off New Caledonia. These, designated carolisterols A - C (1 - 3), are characterized by a polyhydroxycholanic acid moiety, in which the 24-carboxylic acid function is found as an amide derivative of D-cysteinolic acid.*

Extensive studies of starfishes steroid constituents have yielded a large number of steroidal oligoglycosides accompanied by numerous polyhydroxysteroids in both sulphated and non sulphated form[1]. More than eighty polyhydroxysteroids from starfishes have been reported so far[1]. The large majority of them possess a 3β,6α (or β), 8, 15α (or β), 16β-pentahydroxycholestane nucleus, sometime with additional hydroxyl groups at one or more of positions 4β, 5α, 7α (or β) and occasionally 14α. A 26-hydroxyl function is usually present in the side chain, less commonly the side chain is hydroxylated at C-24. All hydroxyl groups are disposed on one side of the tetracyclic nucleus inducing an amphiphilic character in the molecules[2].

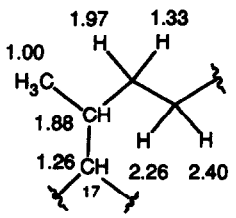
As a part of our continuing investigation of the New Caledonian marine species, we have examined the polar extracts of the starfish *Styracaster caroli* collected at a depth of 2000 m between the islands of Thio and Lifou and wish to report the isolation of three unique polyhydroxysteroids, carolisterols A - C (**1** - **3**).



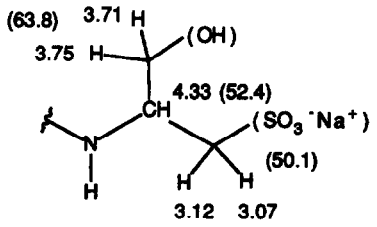
1, carolisterol A R=OH; R'=⁻OH
2, carolisterol B R=OH; R'==O
3, carolisterol C R=H; R'=···OH

Separation of the polar steroids from the aqueous and acetone extracts of *Styracaster caroli* (2 Kg fresh) was achieved by chromatography on a column of Sephadex LH-20, followed by droplet counter current chromatography and reversed phase HPLC to yield carolisterol A (1, 6.0 mg), B (2, 3.3 mg) and C (3, 2.7 mg).

The negative fast atom bombardment (FAB) mass spectrum of carolisterol A (1) exhibited a molecular anion peak at *m/z* 576 [M$^-$], indicating the presence of at least one nitrogen atom in the molecular formula. The IR spectrum contained an absorbance at 1653 cm^{-1}, typical for an amide function, and absorbance at 1200 and 1044 cm^{-1}, consistent with the presence of a sulphonate salt[3]. The ^{1}H NMR spectrum of carolisterol A (1) showed signals at 4.04 m (H-3α), 3.50 t (J= 2.5 Hz, H-6α), 3.78 dd (J= 11.0, 2.5 Hz, H-15β) and 4.10 dd (J= 9.0, 2.5 Hz, H-16α), these latter two coupled to each other by 2.5 Hz, suggesting the presence of a 3β,5α,6β,15α,16β-pentahydroxycholestane tetracyclic nucleus, already found in polyhydroxysteroids isolated from the starfish *Luidia maculata*[4] and *Myxoderma platyacanthum*[5]. The spectrum also contained two methyl singlets for 18- and 19-CH_3 groups at 0.94 and 1.20 ppm and only one methyl doublet (1.00 d, J= 7 Hz). 2D-COSY experiments allowed the connectivities C-1 to C-4, C-6 to C-12 and C-6 to C-17 to be established within the steroidal tetracyclic framework, along with the partial structures (**A**, **B**) shown below.



A



B

The ^{13}C NMR and DEPT spectra contained 27 signals, including one at 176.1 ppm consistent with an amide carbonyl. The complete ^{1}H and ^{13}C NMR assignments are summarized in Table 1. HMBC experiments established the connection between the methylene protons at δ 2.26 and 2.40 (H_2-23) and the carbonyl carbon. Thus, the 3β,5α,6β,15α,16β-pentahydroxycholanic acid structure could be defined for the steroidal moiety 1. HETCORR experiments allowed us to correlate the carbon signals at δ_C 63.8 (CH_2), 52.4 (CH) and 50.1 (CH_2) with their associated proton signals at δ_H 3.71-3.75, 4.33 and 3.12-3.07, respectively (partial structure **B**). An inspection of the literature data suggested the presence of the cysteinolic acid residue linked to the steroidal moiety through an amide functionality. The ^{1}H and ^{13}C NMR spectra reported for cysteinolic acid[6] completely agree with our data. D-cysteinolic acid has recently been isolated from fishes and shellfishes[6] and previously from algae[7-9] and the starfish *Asterina pectinifera*[10]. We propose the D configuration by analogy.

Carolisterol B (2) is the 6-keto analog of carolisterol A (1). The negative FAB mass spectrum of 2 exhibited a molecular anion peak at *m/z* 574 [M$^-$], two mass units shifted relative to 1. In addition to the amide band at 1655 cm^{-1}, the IR spectrum contained a strong band 1715 cm^{-1} providing evidence for a ketone, as confirmed by ^{13}C NMR (δ_C 216.0 ppm). An examination of ^{1}H and ^{13}C NMR spectra immediately indicated the presence of the same cysteinolic acid residue as in 1. The keto function was localized at C-6 by a ^{1}H-^{1}H COSY experiment (Table 1) which correlated the methylene protons α to the keto group, δ 2.33 and 3.01(H_2-7), to H-8 until H_2-23, and comparison of ^{13}C NMR spectrum of 2 with that of 1 (Table 1).

The ^{1}H NMR spectrum of the minor carolisterol C (3) indicated the presence of the same cysteinolic acid residue as in 1 and 2. The negative FAB mass spectrum exhibited a molecular ion peak at *m/z* 560 [M$^-$], corresponding to a tetrahydroxylated saturated cholanic acid linked to the cysteinolic residue. In agreement with a tetrahydroxysteroidal structure, the ^{1}H NMR contained four methine signals at δ 3.50 with the complexity normally observed for a 3β-hydroxyl group, at δ 3.36, in the form of a double triplet (J = 4.0 and 10.5 Hz) characteristic of a 6α-hydroxy group, and at 3.76 dd (J= 11.0, 2.5 Hz) - 4.10 dd (J= 9.0, 2.5 Hz) coupled to each other, already seen in the spectra of 1 and 2 and assigned to the presence of 15α,16β-dihydroxy functions. On this basis we suggest structure 3 for the minor carolisterol C (3).

Table 1. 1H and 13C NMR data for carolisterols A - C (1 - 3) [a]

	1			2		3
C	$^{13}C\delta$	mult[b]	$^{1}H\delta$[c]	$^{13}C\delta$	$^{1}H\delta$	$^{1}H\delta$
1	33.4	CH_2	α 1.62 m	31.3	-	-
			β 1.38 m		-	-
2	31.5	CH_2	α 1.80 m	31.0	-	-
			β 1.53 m		-	-
3	68.2	CH	4.04 m	67.9	3.93 m	3.50 m
4	41.3	CH_2	α 1.60 m	36.6	-	-
			β 2.10 t (13.0)		-	-
5	76.4	C	-	81.0	-	-
6	76.2	CH	α 3.50 t (2.5)	216.0	-	-
			-		-	β 3.36 dt (10.5, 4.0)
7	35.0	CH_2	α 1.90 m	43.1	α 3.01 t (13.5)	-
			β 1.90 m		β 2.33 dd (13.5, 5.4)	-
8	31.0	CH	2.05 m	38.0	-	-
9	46.4	CH	1.47 m	45.7	-	-
10	39.2	C	-	43.4	-	-
11	21.8	CH_2	α 1.42 m	22.2	-	-
			β 1.42 m		-	-
12	41.7	CH_2	α 1.25 m	41.5	-	-
			β 2.00 m		-	-
13	44.5	C	-	44.7	-	-
14	60.6	CH	1.03 m	60.9	-	-
15	84.2	CH	β 3.78 dd (11.0, 2.5)	83.8	β 3.74 dd	β 3.76 dd
16	82.9	CH	α 4.10 dd (9.0, 2.5)	82.7	α 4.10 dd	α 4.10 dd
17	60.1	CH	1.26 m	60.2	-	-
18	14.8	CH_3	0.94 s	14.8	0.90 s	0.91 s
19	17.3	CH_3	1.20 s	14.3	0.83 s	0.89 s
20	30.8	CH	1.82 m	30.9	-	-
21	18.2	CH_3	1.00 d (7)	18.3	1.00 d (7)	0.99 d (7)
22	32.3	CH_2	1.97-1.23 m	32.3	-	-
23	33.8	CH_2	2.40-2.26 m	33.9	2.38-2.28 m	2.36-2.27m
24	176.1	C	-	176.2	-	-
1'	52.4	CH_2	3.12 dd(14.0, 6.0)	52.5	3.13 dd	3.13 dd
			3.07 dd (14.0, 7.0)		3.08 dd	3.07 dd
2'	50.1	CH	4.33 m	50.3	4.32 m	4.33 m
3'	63.8	CH_2	3.75 dd (11.0, 5.5)	63.9	3.75 dd	3.75 dd
			3.71 dd (11.0, 5.5)		3.71 dd	3.71 dd

[a] All spectra are recorded in MeOH-d_4 at 500 MHz; [b] Determined by DEPT and HETCORR experiments; [c] Assignments based on 2D-COSY results.

In view of the anti-HIV activity recently reported for polar sulphated sterols[11,12], the major carolisterol A (1) was tested in the NCI's primary anti-HIV screen and showed no protection against the cytopathic effects of HIV-1.

The proposed structures for carolisterols are a striking new addition to the large number of polyhydroxysteroids which have been isolated from marine sources. No bile acid-type sterols have been isolated from marine sources other than those from fish bile, the unusual 20-epicholanic acid derivatives from the sea pen *Ptilosarcus gurneyi*[13] and two "normal" cholanic acid derivatives from the nudibranch *Aldisia sanguinea cooperi*[14], but never found as polyhydroxylated derivatives.

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