Marine eicosanoids: Occurrence of 8,11,12-trihydroxylated eicosanoic acids in starfishes

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Received 25 April 1991; accepted 31 July 1991

Abstract. The occurrence of 8,11,12-trihydroxyeicosa-5,14,17(Z),9(E)-tetraenoic acid and 8,11,12-trihydroxyeicosa-5,14(Z),9E-trienoic acid in starfish species, i.e. *Patiria miniata, Dermasterias imbricata, Pycnopodia helianthoides, Culcita novaeguinea* and *Nardoa tubercolata* is reported.

Key words. Starfish; marine trihydroxylated eicosanoic acids.

The oxygenation of arachidonic acid and other eicosapolyenoic fatty acids by a variety of cell types results in the formation of several structurally distinct classes of biologically active compounds, such as prostaglandins, thromboxanes, leukotrienes and lipoxins. Leukotrienes and lipoxines are formed by a mechanism which involves initial lipoxygenation of arachidonic acid at the C-5 or the C-15 position, respectively[1].

Recently we have described the occurrence of 8-(R)-HETE (i.e. (8R)-8-hydroxyeicosa-5,11,14(Z),9(E)-tetraenoic acid) in the starfish *Patiria miniata*[2]. This finding is of interest in connection with the biosynthesis of marine prostanoids, which Corey and co-workers[3] recently demonstrated to involve a lipoxygenase type pathway which starts from 8-(R)-HPETE (i.e. (8R)-8-hydroperoxyeicosa-5,11,14(Z),9(E)-tetraenoic acid) and thus differs markedly from the traditional cyclooxygenase oxidation pathway of mammalian prostaglandins. The recent discovery that 8-(R)-HETE specifically triggers starfish oocyte maturation[4] added another reason for interest in this result. 8-(R)-HETE was accompanied in *Patiria miniata* by its 17-didehydroderivative.

P. miniata also gave a trihydroxylated unsaturated C20 fatty acid derivative, a possible further metabolite of 8-(R)-HETE. This derivative was not characterized because of the minute amounts present. Continuing with our systematic search for new active metabolites from echinoderms, we have isolated this trihydroxylated unsaturated fatty acid from *Dermasterias imbricata, Pycnopodia helianthoides, Culcita novaeguinea* and *Nardoa tubercolata*. In this paper we report that this fatty acid is 8,11,12-trihydroxyeicosa-5,14,17(Z),9(E)-tetraenoic acid (**1**), accompanied by its 17-dihydroderivative (**2**), and suggest the 8R,11S,12R configuration for the compounds.

The free fatty acids were obtained by chromatography of aqueous extracts of the animals on Amberlite XAD-2, followed by chromatography of the methanol eluates on a column of Sephadex LH-20 (eluent: methanol-water, 2:1). The steroidal glycosides and the polyhydroxylated sterols were separated from the eicosanoids by droplet counter-current chromatography (dccc), with the solvent system $CHCl_3$-MeOH-H_2O (7:13:8) (ascending mode). The less polar fractions were then submitted to hplc on a $C_{18}\mu$-Bondapack column with methanol-water (1:1) to give compounds **1** and **2**.

Neither **1** nor **2** produced a molecular ion in the electron impact MS. The FAB-MS (negative ion mode) of **1** did have a quasi molecular ion peak at m/z 351 $(M-H)^-$. The ^{13}C NMR spectrum in methanol-d_4 exhibited signals for twenty carbon atoms: one CO (176.7 ppm), eight olefinic methine carbons (136.1, 132.8, 132.5, 131.1, 131.0, 128.2, 127.3 and 126.6 ppm), three hydroxymethine carbons (76.1, 76.0, 73.0 ppm), seven methylene carbons (38.6, 36.3, 31.8, 28.4, 27.6, 26.6, 21.4 ppm) and one methyl carbon (14.5 ppm). These data were consistent with the formula $C_{20}H_{32}O_5$ for the acid **1**. The acid **1** formed an acetonide derivative indicating the presence of a vicinal diol functionality. Analysis of the ^1H NMR spectrum of **1** (table 2) and sequential decoupling experiments al-

Table 1. Yield of compounds **1** and **2** in starfishes

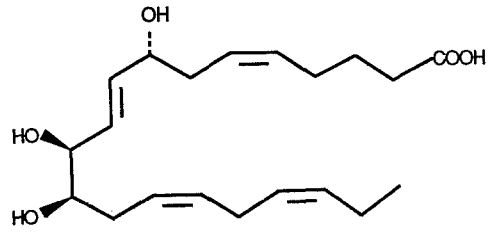
Starfish	Weight (fresh)	1	2
Patiria miniata[a]	(3.5 kg)	trace	
Dermasterias imbricata[a]	(1.2 kg)	2.5 mg	
Pycnopodia helianthoides[a]	(2.2 kg)	4 mg	5 mg
Culcita novaeguinea[b]	(3.8 kg)	1.3 mg	
Nardoa tubercolata[b]	(1.8 kg)	2 mg	

[a] Collected off the Gulf of California, USA. [b] Collected at Zampa, Okinawa.

Table 2. 250 MHz ^1H NMR data for **1** and **2**

H at C	1	2
2	2.20 t (7)	2.20 t(7)
3	1.69 q (7)	1.69 q (7)
4	2.12 m	2.10 m
5–6	5.30–5.50 m	5.30–5.50 m
7	2.32 m	2.32 m
8	4.12 q	4.11 q
9–10	5.78 m	5.78 m
11	4.01 t (4.8)	4.01 t (4.8)
12	3.56 m	3.56 m
13	2.32 m	2.32 m
14–15	5.30–5.50 m	5.30–5.50m
16	2.86 t (6)	-
17–18	5.30–5.50 m	-
19	2.12 m	-
20	1.0 t (7.5)	0.94 t (7.5)

Measured in CD_3OD, chemical shift in ppm; coupling constants in Hertz enclosed in parentheses; t = triplet, q = quartet, m = multiplet.



1

2, 17(18)-dihydro

lowed us to propose the gross structure of 8,11,12-trihydroxyeicosa-5,9,14,17-tetraenoic acid.

The trans geometry of the Δ^9 double bond was verified by a ^{1}H NMR study of the acetonide of **1**. This derivative was prepared by stirring 1 mg of **1** at room temperature for 4 h with 2,2-dimethoxypropane (0,5 ml) and few milligrams of the resin Dowex 50 WX2. The mixture was centrifuged, then evaporated in vacuo, and the residue was analyzed by FAB-MS, m/z 391 $(M-H)^-$ and ^{1}H NMR in CD_3OD at 250 MHz. The C-9 and C-10 protons were clearly doublets of doublets (J = 15, 5.8 Hz) at δ 5.77 and 5.65 ppm, coupled respectively with the triplet at δ 4.65 assigned to H-11 and with the 2H multiplet at δ 4.15 assigned to H-8, and H-12, which coincidentally overlap. The *cis* geometry of the remaining double bonds was assigned by analogy with 8-HETE and comparison of their ^{1}H NMR spectra.

The chemical shifts of the two geminal methyl groups in the ^{1}H NMR spectrum of the acetonide of **1** were quite different (δ 1.37 and 1.50), and this means that the substituents were *cis* to each other on the acetonide ring. On the basis of the data above, compound **1** is an erythro 8,11,12-trihydroxyeicosa-5,14,17(Z),9(E)-tetraenoic acid. We assume the 8R stereochemistry by analogy with 8-(R)-HETE. Moreover, of the two possible configurations, 8(R), 11(R), 12(S) and 8(R), 11(S), 12(R), we prefer the latter, because the ^{1}H NMR signals for the C-8/C-13 substructure are very similar to those reported for the analogous substructure of malyngic acid, 9(S), 12(R), 13(S)-trihydroxyoctadeca-10(E), 15(Z)-dienoic acid (same relative configuration as **1**), isolated from *Lyngbya majuscula*[5].

The 8,11,12-trihydroxyeicosa-5,9,14,17-tetraenoic acid has been previously described, and named trioxilin A_4, as a hydrolysis product of the epoxilin A_4 (i.e. 8-hydroxy-11,12-epoxyeicosa-5,14,17(Z),9(E)-tetraenoic acid), which is obtained by treatment of 12-hydroperoxyeicosa-5,8,10,14,17-pentaenoic acid with bovine emine[6].

The second trihydroxylated eicosanoic acid (**2**) from starfishes was characterized as the 17(18)-dihydroderivative of **1**, on the basis of FAB mass spectrometry, m/z 353 $(M-H)^-$, ^{1}H NMR (table 2) and comparison with **1**. The biological role of these trihydroxylated eicosanoic acids in starfishes remains to be discovered.

Acknowledgments. This work was supported by Progetto Finalizzato Chimica Fine II, C.N.R. (Rome). We are grateful to Professor William Fenical, Scripps Institution of Oceanography, La Jolla, California, USA, for collection of the starfishes from the Gulf of California and Professor Tatsuo Higa, University of the Ryukyus, Okinawa, Japan, for collection of the starfishes from Zampa, Okinawa.

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