Pergamon

Tetrahedron Letters, Vol. 37, No. 17, pp. 2959-2962, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00454-6

New Biologically Active Pectinoacetal-Related Sterols from the Gorgonian *Ctenocella sp.*

X.C. Fretté*, J.F. Biard, C. Roussakis and J.F. Verbist
Institut des Substances et Organismes de la Mer (ISOMer), SMAB[1], BP 1024, 44035 Nantes Cedex 01, France

J. Vercauteren and N. Pinaud
Laboratoire de Pharmacognosie[2], Université de Bordeaux II, Bât 3A, 146 rue Léo Saignat, 33076 Bordeaux, France

C. Débitus
ORSTOM, Centre de Nouméa, BP A5, Nouméa Cedex, Nouvelle Calédonie

Abstract : Based on a bio-assay guided fractionation, a new sterol has been isolated as an equilibrium of isomers **1** with interesting cancer cell antiproliferative properties from a yet undescribed species of the genus *Ctenocella sp.* The structure of this sterol has been inferred from the acetyl derivative **2**, on the basis of spectral study. Copyright © 1996 Elsevier Science Ltd

Gorgonians are a rich source of biologically active products among which are prostaglandins[3,4], diterpenes[3,5-7] (mainly briaranes and cembrenes), polymethylene butenolides[8] and sterols[9]. Several compounds have shown a broad range of potent bioactivities, mainly antimicrobial[10], anticancer[11,12], and antiinflammatory[6,13]. *Ctenocella* belongs to the little investigated Ellisellidae family (Cnidaria) which yielded usual sterols (*Ellisella paraplexauroides*[9]), juncins A-F (*Junceella juncea*[14]), junceellolides A-D (*Junceella fragilis*[13]), junceellin and praelolide (*Junceella squamata*[13]), gemmacolides A-F (*Junceella gemmacea*[15]) while *Ctenocella pectinata* yielded pectinoacetals A-C[16].

Extraction. The gorgonian, *Ctenocella sp*[17] (2.3 kg, dry weight), has been collected off the New Caledonian coast in spring 1993 at a depth of 20 meters by the local ORSTOM branch. Samples were deep-freeze dried, blended and exhaustively extracted by 95% ethanol. The crude extract obtained was submitted to screening tests aimed *inter alia* at selecting organisms containing products inhibiting cancer cell proliferation (KB[18] and NSCLC[19] clones). Since a positive result was observed (IC_{50}=16.3 µg/mL on NSCLC and 3.9 µg/mL on KB cells), the crude extract was submitted to solvent partition (hexane, CCl_4, then CH_2Cl_2 *vs* water:methanol). As the Bioassay guided fractionation showed the most active fraction was the carbon tetrachloride soluble fraction, it was flash chromatographed over diol phase with elutions by CH_2Cl_2:MeOH (98:2) to (70:30). The first fraction (98:2) was then submitted to HPLC fractionations on diol phase with isocratic elution by 1) CH_2Cl_2:MeOH (98:2), 2) hexane:CH_2Cl_2 (60:40), and 3) hexane:dioxane (76:24).

The third HPLC allows separation of two close peaks (UV and RI detections), both of which reform the previous 2-peak equilibrium within 3 hours following their separation as can be checked by a control HPLC injection. Since the IR spectrum of the mixture showed an alcohol function, an acetylation was undertaken allowing isolation of a single product **2** as a colourless oil ($5\ 10^{-4}$% of dry weight sample).

Structural elucidation. On the basis of high resolution positive-ion LSIMS the empirical formula $C_{29}H_{42}O_5$[20], was established for sterol **2**, thus revealing 9 Degrees of Unsaturation (DU).

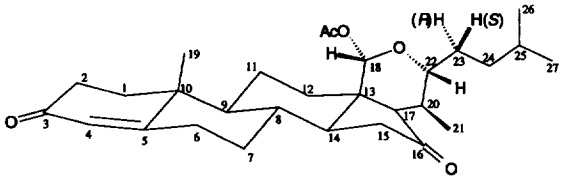

Fig. 1 : Acetyl derivative **2**

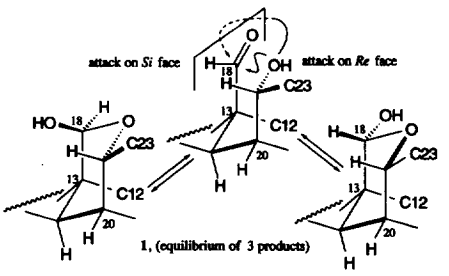

Fig. 2 : Partial spatial view of product **1**

The carbon content was corroborated by *J*-modulated ^{13}C NMR showing resonances that are readily assigned to an acetyl group (169.19 and 21.54 ppm), an enone system (199.11, 124.24 and 169.8 ppm), a pentacyclic keto group (216.41 ppm), an hemiacetal carbon (93.64 ppm) and an oxygenated methine(79.86ppm). In addition, this spectrum showed signals for six quaternary, nine methine, nine methylene and five methyl carbons. ^1H NMR data displayed 3 downfield shifted signals attributed to one ethylenic proton (H-4), one acetal proton (H-18) and one for a proton geminal to an oxygen (H-22). The UV spectrum displays a characteristic band at 242 nm (ε=17400, in $CHCl_3$) due to the enone system. The IR spectrum exhibits bands for the enone system (1672 cm^{-1}: ν C=O, 1617 cm^{-1}: ν C=C), the pentacyclic oxo group (1739 cm^{-1}), the acetyl group (1225 cm^{-1}) and the ether bond (1049 cm^{-1}). Therefore 4 DU are directly attributed.

^{13}C NMR data, compared to ^{13}C chemical shifts of numerous sterols, matched with the basic skeleton of testosterone for rings A and B, while they fitted better those of 5α-androstan-16-one[21] for rings C and D. Our experimental values are also very much in accordance with those of Roussis and Fenical, for pectinoacetals A-C[16]. However, in both cases there were discrepancies in carbon chemical shifts of ring D, and side chain. These facts led us to a sterol skeleton, with both C-18 and side chain modified compared to the above mentioned products. The side chain shows the characteristic isopropyl end group (IR : twin peaks at 1366 and 1376 cm^{-1}, ^{13}C NMR : 22.25, 22.81 and 27.91 ppm, ^1H NMR : 0.85, 0.87 and 1.51 ppm) as well as the C-21 methyl (14.96 ppm). C-18 chemical shift is that of an hemiacetal and this is confirmed by an HMBC correlation between H-18 and the carboxyl group of the acetate. HMBC reveals further connectivities between C-18,H-22 and C-22,H-18. With regard to C-18 and C-22 downfield shifts, it was considered that both carbons are connected through an ether link. All 9 DU are now accounted for, and we now have the assignments of all carbon chemical shifts.

Even though ^1H NMR spectra of sterols have been considered until recently as an "undecipherable, broad, almost featureless hump between ~1 and 2.5 ppm"[22], the systematic use of 500 MHz and 2D NMR allow nearly complete ^1H and ^{13}C assignments. We have employed these techniques to support our structure proposal with a stereochemical insight, *i.e.* conformation of the steroid skeleton and of the tetrahydropyran extra-ring. As can be seen from table 1, only eleven protons afford accurate coupling constant readings. All other signals overlap and cannot provide such information. However, signal width[22] provides an indirect *J* evaluation (table 1) thus allowing a full δ ^1H assignment tentative. On these asumptions we are able to propose the structure shown in figure 1.

Table 1. ^{1}H and ^{13}C NMR Data of Acetylated Sterol **2** ($CDCl_3$, 500 MHz)

C	^{13}C	HMQC	H	COSY	HMBC	*J* or signal width (Hz)	Pattern designation
1	35.42	2.04	1α	2.44, 2.36, 1.73	199.11, 38.61, 33.84, 17.37	13.4, 13.9, 4.9	1g, 1aa, 1ae
		1.73	1β	2.44, 2.36, 2.04	199.11, 53.52, 38.61, 33.84, 17.37	13.4, 4, 3.6	1g, 1ae, 1ee
2	33.84	2.44	2β	2.36, 2.04, 1.73	199.11, 124.24, 35.42	38[c]	1g1aa1ae
		2.36	2α	2.44, 2.04, 1.73	124.24	21.7[c]	1g1ae1ee
3	199.11	-	-	-	-		
4	124.24	5.76	4	2.44 - 2.32, 1.17	38.61, 33.84, 32.35		
5	169.80	-	-	-	-		
6	32.35	2.43	6β	2.32, 1.85, 1.13	169.8, 32.18	38[c]	1g1aa1ae
		2.32	6α	2.43, 1.85, 1.13	169.8	21.8[c]	1g1ae1ee
7	32.18	1.85	7β	2.43, 2.32, 1.13	-	26.4[c]	1g1ae1ee
		1.13	7α	2.43, 2.32, 1.85	50.08, 34.38	43.3[c]	1g2aa1ae
8	34.38	1.87	8β	1.16, 1.61		39.8[c]	3aa1ae
9	53.52	1.16	9α	1.87, 1.68, 1.57	50.08, 32.18, 34.38, 21.29, 17.37	31.1[c]	2aa1ae
10	38.61	-	-	-	-		
11	21.29	1.68	11α	2.52, 1.57, 1.23, 1.16	-	19.2[c]	1g1ae1ee
		1.57	11β	2.52, 1.68, 1.23, 1.16	53.52, 46.01, 31.94	43.7[c]	1g2aa1ae
12	31.94	2.52	12β	1.68, 1.57, 1.23	46.01	13, 4, 3.1	1g, 1ae, 1ee
		1.23	12α	6.06, 2.52, 1.68, 1.57	93.64, 46.01, 21.29	35[c]	1g1aa1ae
13	46.01	-	-	-	-		
14	50.08	1.61	14α	2.27, 2.23, 1.87	46.01, 34.38	33.6[c]	2aa1ae
15	38.85	2.27	15α	2.23, 1.89, 1.61	216.41, 50.08, 34.38	18, 8.4	1g, 1ae
		2.23	15β	2.27, 1.61	216.41, 50.08, 34.38	18, 14	1g, 1aa
16	216.41	-	-	-	-		
17	60.78	1.89	17α	1.61, 1.95	216, 93.64, 79.86, 46, 33.79, 31.94, 14.96	4.2	1ae
18	93.64	6.06	18	1.23	169.19, 79.86, 60.78, 50.08, 46.01, 31.94		
19	17.38	1.17	19	5.76	169.8, 53.52, 38.61, 35.42		
20	33.79	-[a]	20	3.4, 1.25, 1.89	216.41, 93.64, 60.78, 46.01, 31.94, 14.96	10.1, 7.2, 4.2	1aa, 1ae, fr
21	14.96	1.25	21	1.95	79.86, 60.78, 33.79	7.2	fr
22	79.86	3.4	22	1.95, 1.6, 1.35	-	10.1, 2.9	1aa, H22-23
23	31.79	1.6	23*r*	3.4, 1.35, 1.21	-	21[c,e]	d
		1.35	23*s*	3.4, 1.35, 1.21	27.91, 22.81, 22.25	32[c,e]	d
24	34.54	-[b]	24[f]	1.6, 1.51, 1.35	31.79, 27.91, 22.81, 22.25	d	
25	27.91	1.51	25	1.35, 0.87, 0.85	22.81, 22.25	d	
26	22.25	0.85	26	1.51	34.54, 27.91, 22.81, 22.25	7	fr
27	22.81	0.87	27	1.51	34.54, 27.91, 22.81, 22.25	7	fr
Ac	169.19	-	-	-	-		
Ac	21.54	2.14	Ac	-	93.64		

a, [b]Assignments not visible in the spectrum, deduced from HMBC (1.95 ppm)[a] and (1.21 ppm)[b].
[c]Signal width, [d]not measurable or interpretable accurately, [e]interchangeable assignments, [f]two protons.
g: geminal; aa: axial-axial; ae: axial-equatorial; ee: equatorial-equatorial; fr: free rotation.

The clearly visible connectivity between H-18 and H-12*a*, implying a planar shape to allow a W ^{4}J coupling, and the antiperiplanar coupling constant (J=10.1 Hz) displayed by H-22 can only be met if the tetrahydropyran ring is in chair conformation $^{20}C_{18}$, *i.e.* the anomeric proton is α to the plane of the tetrahydropyrannyl ring. Moreover, in this conformation, the methyl-21 is located in the shielding cone of the carbonyl group, hence justifying its low chemical shift (14.96 ppm).

This structure underlies the two-peak equilibrium observed during the final purification step. The extra-ring is generated by a lactol cyclisation between C-18 and C-22 involving an attack of the OH-22 on the *Re* face of the aldehyde we assume to be present at C-18[23]. An attack on the aldehyde *Si* face would of course lead to its epimer, but upon acetylation, prefered isomer **2** is the only formed product.

The previous study carried out on *Ctenocella* targeted pectinoacetals A, B and C on their UV absorbance. Our study afforded pectinoacetal-related sterols based on biological activity. These sterols have only been reported in *Ctenocella* and could be considered as specific markers of this genus. Equilibrium-product **1** showed cancer cell antiproliferative properties (IC_{50} = 0.23 µg/mL on KB cells, IC_{50} = 2.9µg/mL on NSCLC-N6 cells). A more detailed pharmacological study on **1** and **2** is under publication.

REFERENCES AND NOTES

1. S.M.A.B. Research Group (Substances Marines à Activité Biologique) "JE D.R.E.D. JE 257".
2. G.E.S.N.I.T. (Groupe d'Études des Substances Naturelles à Intérêt Thérapeutique), "EA 491 MESR"
3. Faulkner, D.J. *Nat. Prod. Rep.* **1988,** *5*, 613-663
4. Corey, E.J.; Matsuda, S.P.T. *Tetrahedron Lett.*, **1987**, *28*, 4247-4251
5. Faulkner, D.J. *Nat. Prod. Rep.* **1991**, *8*, 97-147
6. Look, S.A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363-3370
7. Shin, J.; Fenical, W. *J. Org. Chem.* **1991**, *56*, 3153-3158
8. Rodriguez, A.D.; Ramirez, C. *J. Nat. Prod.* **1994**, *3*, 339-347
9. Miralles, J.; Galonnier, R.; Njinkoue, J.M.; Samb, A.; Gaydou, E.M.; Kornprobst, J.M. *Comp. Biochem. Physiol.* **1992**, *4*, 1039-1041
10. McEnroe, F.J.; Fenical, W. *Tetrahedron* **1987**, *34*, 1661-1664
11. Faulkner, D.J. *Nat. Prod. Rep.* **1984**, *6*, 551-598
12. Higa, T.; Tanaka, J.; Tsukitani, Y.; Kikuchi, H. *Chem.Lett.* **1981**, 1647-1650
13. Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *6*, 1633-1638
14. Isaacs, S.; Carmely, S.; Kashman, Y. *J. Nat. Prod.* **1990**, *53*, 596-602
15. He, H.; Faulkner, D.J. *Tetrahedron* **1991**, *47*, 3271-3280
16. Roussis, V.; Fenical, W.; Harvala, C. *Experientia* **1993**, *49*, 265-267
17. Species is presently under study and will be described by Dr M. Grasshoff. A voucher specimen is kept by ORSTOM in Nouméa - New Caledonia.
18. Geran, R.I.; Greenberg, N.H.; Macdonald, M.M.; Schumaker, A.M.; Abbott, B.J. *Cancer Chemother. Rep.*, **1972**, *2*, 7-57
19. Roussakis, C.; Dabouis, G.; Gratas, C.; Audouin, A.F. *5th N.C.I-E.O.R.T.C. Symposium on new drugs in cancer therapy. Amsterdam, Abstract.* **1986**, *248*
20. MH^+ observed = 471.309156; required MH^+ = 471.311050
21. Blunt, J.W; Stothers, J.B. *Org. Magn. Reson.* **1977**, *9*, 439-464
22. Croasmun, W.R.; Carlson, R.M.K. *Methods in Stereochemical Analysis. 9. Two-Dimensional NMR Spectroscopy. Applications for Chemists and Biochemists*; Edited by Coasmun W.R. and Carlson R.M.K.; VCH Publishers: New York, 1987; pp. 387-424
23. Aldehyde function seen in NMR spectra of **1**, in ^1H NMR : 9.8 ppm, in ^{13}C NMR : 207 ppm, both C and H showing a connectivity in HMQC-HOHAHA. An HMBC connectivity between H-aldehyde and C-13 is also visible.

(*Received in France* 9 *February* 1996; *accepted* 6 *March* 1996)