Tetrahedron Letters, Vol. 33, No. 8, pp. 1097-1100, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

The First Occurrence of Polyhydroxylated Steroids with Phosphate Conjugation from the Starfish *Tremaster novaecaledoniae*

Francesco De Riccardis, Maria Iorizzi, Luigi Minale* and Raffaele Riccio

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II"
Via Domenico Montesano 49, 80131 Napoli, Italy

Cécile Debitus

ORSTOM, Centre de Nouméa, BP. A5, Nouméa, New Caledonia.

Abstract: Three steroid constituents have been isolated from the starfish *Tremaster novaecaledoniae* (Jangoux 1982) collected at a depth of 530 m off New Caledonia. Compounds **1** - **3**, designated as tremasterol A - C, are characterized by the presence of 3β-O-sulphated, 6α-O-phosphated and 16β-O-acetylated groupings on a steroidal skeleton. In compound **1** the monophosphate residue is further linked to 1'-glucose (1'-glucose tetracetate in **2** and 1'-glucose-6'-acetate in **3**).

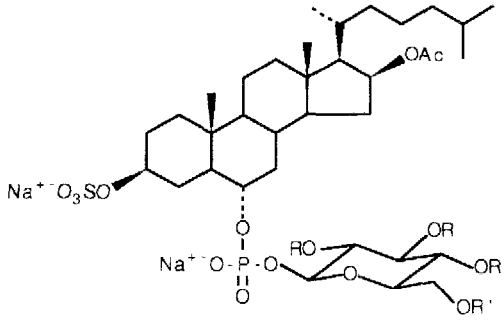
It is known that there are three groups of steroidal oligoglycosides in starfishes: sulphated steroidal penta- and hexa-glycosides ("asterosaponins"), steroidal cyclic glycosides (so far only found in two species of the genus *Echinaster*), and glycosides of polyhydroxysteroids consisting of a polyhydroxysteroid with one or two sugar units, which are found in both sulphated and non sulphated form. Often these saponins are accompanied by small amounts of polyhydroxylated steroids, also occurring in both sulphated and non-sulphated form[2,3]. In our continuing investigation of the New Caledonian marine species we have examined the polar extracts of the starfish *Tremaster novaecaledoniae* (Jangoux 1982), which is a "living fossil" species[4], collected at depth of 530 m off New Caledonia, and wish to report the first isolation of phosphated polyhydroxysteroids from a natural source. Compounds **1** - **3** are also sulphated.

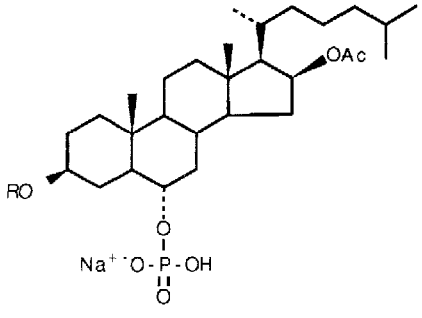
Compounds were isolated and purified by chromatography on Sephadex LH-60 (methanol-water 2:1 as eluant) of the methanol soluble portion of the acetone extracts, followed by droplet counter current chromatography [DCCC, butan-1-ol : acetone : water (3:1:5); descending mode] and HPLC (μ-Bondapak C_{18}, 30 cm x 7.8 mm i.d.; methanol-water 1:1 as eluant).

Tremasterol A (**1**), $[\alpha]_D$ = +40° (c 0.5, MeOH) exhibited, in its negative-ion FAB mass spectrum, molecular ion species at m/z 803 (80%) and 781 (40%), corresponding to $[M_{Na}]^-$ and $[M_H]^-$, respectively, and intense fragment ion peaks at m/z 641 (100%) and 619 (90%) arising by loss of the glucosyl residue (= 162 m. u.). Fragment ion peaks at m/z 539 (20%) and 521 (50%) were interpreted as loss of SO_3 from m/z 619 and $NaHSO_4$ from m/z 641. The presence of a steroidal skeleton was deduced from ^{1}H NMR methyl proton signals (Table 1) and one sugar unit (identified as glucose; anhydrous acid methanolysis) would be also considered for the signals in the 3.28 - 3.68 region and the anomeric one at δ 4.89.

The presence of an acetoxy group was shown from IR (ν_{max} 1735 cm^{-1}), ^{1}H NMR (δ_H 2.02 s, 3H) and ^{13}C NMR (δ_C 172.4 and 21.2 ppm) data. In addition to the sugar moiety and the acetoxy group, the ^{13}C NMR spectrum revealed carbon signals in agreement with a trioxygenated cholestane structure (Table 1). 2D-COSY experiment allowed the sequential assignement of the resonances for the glucosyl residue and also the establishment of the connectivities C-1 to C-8 and C-15 to C-21 within the steroidal framework. The multiplet at δ 4.24 had the complexity normally seen for a 3β-oxygenated group and its downfield chemical shift, virtually identical to that

seen in the spectrum of the 5α-cholestan-3β-yl sulphate, suggested a sulphate group located there. The multiplet at δ 4.06 appearing as a dddd (J = 9.5, 9.5, 7.5 and 4.5 Hz), was assigned to an axial proton (H–6β) in a cholestane skeleton. The axial proton associated with the 6α-hydroxyl group is usually seen as a triple doublet[5] (J = 9.5 and 4.5 Hz), and the shape of the 6β-proton signal in **1** was suggestive for the presence of a phosphate (typical $J_{H\text{-}C\text{-}O\text{-}P} = ca\ 8.4$ Hz)[6], to which the β-glucopyranosyl residue is glycosidally linked. This was confirmed by the proton noise decoupled ^{13}C NMR spectrum, in which 1', 2', 5 and 6 carbons appear as doublets with $J_{P\text{-}O\text{-}C} = 5.5$ Hz and $J_{P\text{-}O\text{-}C} = 7.4$ Hz. The presence of a phosphate group linking C-6 of the steroid and C-1' of glucose was definitively confirmed by ^{31}P NMR spectrum, which showed a triplet (J = 7.5 Hz) signal at 3.54 ppm downfield from external standard (H_3PO_4 85% in D_2O), converted into doublets on irradiation at δ_H 4.06 (H-6) and at δ_H 4.89 (H-1'). The ^{13}C chemical shifts of the glucosyl residue were also compatible with those of reference β-O-glucopyranose-1-phosphate[7]. Under very mild acid treatment (reverse phase HPLC using 0.1% CH_3CO_2H in 60% *aq.* methanol) glucose was removed giving **1a**[8], negative-ion FAB mass spectrum, m/z 643 $[M_{Na}]^-$ and m/z 621 $[M_H]^-$. The presence of the sulphate group at C-3 was confirmed by solvolysis of **1** in a dioxane-pyridine mixture, affording the desulphated derivative **1b**[9], negative-ion FAB mass spectrum, m/z 541 $[M_H]^-$, $\delta_{H\text{-}3}$ 3.50 m (4.24 in **1**); $\delta_{C\text{-}3}$ 72.0 ppm (79.9 ppm in **1**). The remaining acetoxy function was located at the 16 position from the chemical shift of the H-16 signal (δ 5.27 m), which showed coupling to signals at δ 2.42 (H-15), 1.32 (H-17) and 1.15 (H-15) ppm in the 2D-COSY spectrum. The β-configuration was deduced from the downfield shift of the 18-methyl protons to





1 R = R' = H

2 R = R' = Ac

3 R = H, R' = Ac

1a R = $SO_3^-Na^+$

1b R = H

δ 0.93, requiring the presence of a 1,3-*syn* interaction, and confirmed by the ^{13}C NMR signal at δ 31.3 ppm assigned to C-20, significantly upfield relative to the model 5α-cholestan-3β-ol[10] (δ_C 36.0 ppm), as expected upon introdution of a substituent at 16β-position.

Therefore, based on the above results, tremasterol A possesses structure **1**, that is 5α-cholesta-3β,6α,16β-triol-3-sulphate,6-(glucose-1')-phosphate,16-acetate.

Tremasterol B (**2**) is the 2',3',4',6'-tetraacetate derivative of **1**. The negative-ion FAB mass spectrum showed molecular anion species at m/z 971 (100%) $[M_{Na}]^-$ and 949 (20%) $[M_H]^-$ accompanied by a fragment ion peak at m/z 641 (25%), corresponding to the loss of a glucosyl tetraacetate residue (330 m.u.) from m/z 971. In addition to the signals for the aglycone already observed in the spectrum of **1**, the ^1H NMR spectrum of **2**[11] contained four more methyl singlets in the 1.99-2.09 region (four acetate groups) and the sugar signals were seen downfield shifted to δ 3.97 (m, H-5'), δ 4.22 (dd, J = 12.5 and 2.5 Hz) - 4.36 (dd, J = 12.5, 5.0 Hz, -CH_2-OAc) and as overlapping signals in the 4.9-5.3 ppm region (H-1', H-2', H-3' and H-4'). The ^{13}C NMR chemical shifts of the glucosyl tetraacetate residue in **2** were compatible with those in references[7]. Reverse phase HPLC using 0.1% CH_3CO_2H in 60% *aq.* methanol resulted in the removal of glucose tetraacetate affording **1a** and, upon solvolysis in a dioxane-pyridine mixture, **2** afforded the desulphated steroid **1b**.

Table 1. NMR data (CD_3OD) of tremasterol A (**1**).

Carbon	^{13}C NMR[a] (ppm)	Proton	^1H NMR[b] (ppm)
1	37.0	1α, 1β	1.12 m, 1.82 m
2	29.5	2α, 2β	2.07 m, 1.61 m
3	79.9	3α	4.24 m
4	31.0	4α, 4β	2,60 m, 1.50 m
5	52.0*		1.30 m
6	76.0*	6β	4.06 dddd (9.5,9,5,7.5,4.5)
7	40.9	7α, 7β	1.08 m, 2.35 m
8	35.1		1.65 m
9	55.5		
10	37.6		
11	22.0		
12	41.1		
13	44.1		
14	55.1		
15	35.8	15α, 15β	2.42 m, 1.15 m
16	76.6	16α	5.27 m
17	61.6		1.32 m
18	13.2		0.93 s, 3H
19	13.9		0.92 s, 3H
20	31.3		1.88 m
21	18.6		1.00 d (7), 3H
22	38.4		
23	25.3		
24	40.6		
25	29.0		
26	23.0		0.91 d (7), 3H
27	22.8		0.91 d (7), 3H
$\underline{C}H_3CO$-	21.2	$C\underline{H}_2CO$-	2.02 s, 3H
$CH_3\underline{C}O$-	172.4		
1'	99.3*		4.89 t (7.5)
2'	76.0*		3.28 dd (9.5,7.5)
3'	77.8		3.45 t (9.5)
4'	71.8		3.27 t (9.5)
5'	78.4		3.44 m
6'	63.0		3.68 dd (11.2,6.2)-3.91 dd (11.2,2.5)

[a] At 62.9 MHz; values relative to $^{13}CD_3OD$ (δ_C = 49 ppm).
[b] At 500.13 MHz by ^1H-^1H correlation spectroscopy; chemical shifts referred to CHD_2OD (δ_H = 3.34), J (in Hz) are given in parenthesis.
*Signals appear as doublets in the proton noise decoupled spectrum.

Tremasterol C (**3**) [negative-ion FAB mass spectrum: m/z 845 (30%) $[M_{Na}]^-$, 823 (100%) $[M_H]^-$ and 619 (loss of the glucosyl-O-acetate residue from m/z 823)] is a monoacetate derivative of the steroid **1**. On very mild acid treatment **3** afforded **1a** whereas on solvolysis in a dioxane-pyridine mixture it gave **1b**. The acetate group on **3** was located at C-6 of the glucopyranose moiety from the ^1H NMR spectrum[12], which showed the C-6 H_2 signals downfield shifted to δ_H 4.23 (dd, J = 12.5 and 5 Hz) and 4.49 (dd, J = 12.5, 2.5 Hz).

Acknowledgements.This contribution is part of the project SMIB (Substances Marines d'Intéret Biologique), ORSTOM-CNRS, Nouméa, New Caledonia. Chemical work was supported by the "Ministero dell'Università e della Ricerca Scientifica e Tecnologica", MURST, ROME. We thank Professor M. Jangoux (Université Libre de Bruxelles) for the identification of the starfish. FAB-MS spectra were provided by "Servizio di Spettrometria di Massa del C.N.R. e dell'Università di Napoli", the assistance of the staff is gratefully acknowledged.

REFERENCES AND NOTES

1 Present address: Università degli Studi del Molise, Facoltà di Agraria - 86100 - Campobasso, Italy.

2 Minale, L.; Riccio R.; Pizza C.; Zollo F. *Natural Product and Biological Activities*, NAITO Foundation Symposium; H. Imura, T. Goto, T. Murachi and T. Nakajima Eds.; University of Tokyo Press, Elsevier Science, **1986**, p. 39.

3 Stonik,V. A.; Elyakov, G. B. *Biorganic Marine Chemistry*; Scheuer P. J. Ed.; Springer Verlag, New York,**1988,** *2*, p. 43.

4 M. Jangoux, personal information.

5 Bridgeman, J. E.; Cherry, P. C.; Clegg, A. S.; Evans J. M.; Jones, Sir Ewart R. H.; Kasal, A.; Kumar, V.; Meakins, G. D.; Morisawa, Y.; Richards, E. E. Woodgate, P.D. *J. Chem. Soc. (C)* **1970**, p. 250.

6 Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Spectral Data for Structure Determination of Organic Compounds*, Springer Verlag, **1989**, p. H360.

7 Breitmaier, E.; Voelter, W.; *Carbon-13 NMR Spectroscopy*, Third Edition, VCH Publisher, **1987**, p. 388.

8 Spectral data of **1a**: ^{1}H NMR (CD_3OD): δ_H = 0.90 (6H, d, J = 7 Hz, CH_3-26 and CH_3-27), 0.91 (3H, s, CH_3-19), 0.93 (3H, s, CH_3-18), 1.00 (3H, d, J = 7 Hz, CH_3-21), 2.02 (3H, s, CH_3CO-), 4.06 (1H, dddd, J = 9.5, 9.5, 7.5 and 4.5 Hz, H-6), 4.25 (1H, m, H-3) and 5.25 (1H, m, H-16) ppm.

9 Spectral data of **1b**: ^{1}H NMR (CD_3OD): δ_H = 0.89 (6H, d, J = 7 Hz, CH_3-26 and CH_3-27), 0.91 (6H, s, CH_3-18 and CH_3-19), 1.00 (3H, d, J = 7 Hz, CH_3-21), 2.03 (3H, s, CH_3CO-), 3.50 (1H, m, H-3), 3.98 (1H, dddd, J = 9.5, 9.5, 7.5 and 4.5 Hz, H-6) and 5.25 (1H, m, H-16) ppm. ^{13}C NMR (CD_3OD); δ_C = C-1: 38.6, C-2: 31.4, C-3: 72.0, C-4: 33.3, C-5: 52.1*, C-6: 76.7*, C-7: 41.0, C-8: 35.1, C-9: 55.6, C-10: 37.0, C-11: 22.1, C-12: 41.4, C-13: 44.0, C-14: 55.3, C-15: 35.8, C-16: 76.7, C-17: 61.7, C-18: 13.2, C-19: 13.9, C-20: 31.8, C-21: 18.6, C-22: 38.5, C-23: 25.4, C-24: 40.6, C-25: 29.0, C-26: 23.0, C-27: 22.8, $\underline{C}H_3CO$: 21.2, $CH_3\underline{C}O$: 172.3.

10 Blunt, J. W.; Stothers, J. B. *Org. Magn. Res.*, **1977**, *9*, p. 439.

11 Spectral data of **2**: ^{1}H NMR (CD_3OD): δ_H = 0.90 (6H, d, J = 7 Hz, CH_3-26 and CH_3-27), 0.92 (3H, s, CH_3-19), 0.93 (3H, s, CH_3-18), 1.00 (3H, d, J = 7 Hz, CH_3-21), 1.99 (3H, s, CH_3CO-), 2.02 (6H, s, CH_3CO-), 2.08 (3H, s, CH_3CO-), 2.09 (3H, s, CH_3CO-), 4.06 (1H, dddd, J = 9.5, 9.5, 7.5 and 4.5 Hz, H-6), 4.24 (1H, m, H-3) and 5.27 (1H, m, H-16) ppm. ^{13}C NMR (CD_3OD); δ_C = C-1': 96.9*, C-2': 73.2*, C-3': 73.4, C-4': 69.6, C-5': 73.5, C-6': 63.3, $\underline{C}H_3CO$-: 20.6 (x2), 20.8, 21.0 ppm.

12 Spectral data of **3**: ^{1}H NMR (CD_3OD): δ_H = 0.90 (6H, d, J = 7 Hz, CH_3-26 and CH_3-27), 0.91 (3H, s, CH_3-19), 0.93 (3H, s, CH_3-18), 1.00 (3H, d, J = 7 Hz, CH_3-21), 2.02 (3H, s, CH_3CO-), 2.10 (3H, s, CH_3CO-), 4.05 (1H, dddd, J = 9.5, 9.5, 7.5 and 4.5 Hz, H-6), 4.24 (1H, m, H-3) and 5.26 (1H, m, H-16) ppm.

* Signals appear as doublets in the proton noise decoupled spectrum.

(Received in UK 4 December 1991)