Tetrahedron Letters,Vol.23,No.28,pp 2899-2902,1982 0040-4039/82/282899-04$03.00/0
Printed in Great Britain

STARFISH SAPONINS. PART 8 . STRUCTURE OF NODOSOSIDE, A NOVEL TYPE OF STEROIDAL GLYCOSIDE FROM THE STARFISH *PROTOREASTER NODOSUS*†

R. Riccio
Istituto di Chimica M.I.B.del C.N.R., Arco Felice, Naples, Italy

L. Minale, C. Pizza and F. Zollo
Istituto di Chimica Biorganica, Università, Via Rodinò n.22, Naples, Italy

and J. Pusset
Laboratoire des Plantes Mèdicinales, C.N.R.S., B.P. 613, Noumèa, New Caledonie

Abstract.- A novel steroidal glycoside has been isolated from the starfish Protoreaster nodosus. The structure includes a 3β,5α,6β,8β,15α,24ξ-hexahydroxysteroidal moiety and a sugar moiety [2-O-methyl-β-D-xylopyranosyl-(1 → 2)-α-L-arabinofuranosyl]which is glycosidically attached at C-24 of the aglycone.

Saponins from starfish are steroidal glycosides and since now two structural types have been encountered. The first one, recognized for long time, is characterized by steroidal aglycones possessing a 3β,6α-diol pattern and a 9,11-double bond; the oligosaccharide moiety is attached at C-6 and a sulphate residue is at C-3[2]. The second structural type, recently discovered in two species of the genus *Echinaster*, has a number of unusual features: a Δ^7,3β,6β-dihydroxysteroidal moiety, there is no sulphate group and, most remarkably, the carbohydrate chain is cyclized between C-3 and C-6 of the aglycone[1,3].

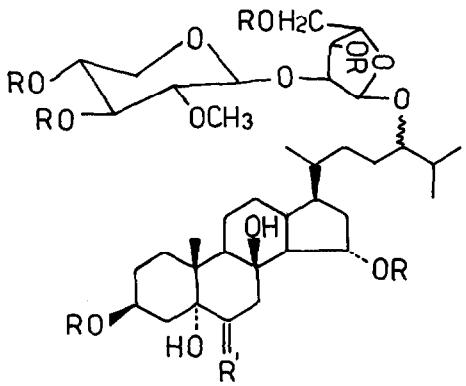
We now report the occurrence in the Pacific starfish *Protoreaster nodosus* of a steroidal glycoside, nodososide (*1*), which is of a completely new type. This material was obtained in 0.003% yield (dry weight basis) from the methanol extract of the lyophilized "starfish" collected off Noumèa, Nouvelle Caledonie, by SiO_2 short column chromatography ($CHCl_3$ and increasing MeOH content to 40%) followed by preparative LC (prepak 500 SiO_2, 30% MeOH/$CHCl_3$) and eventually reversed phase HPLC (C_{18}μ-bondapak, 35% H_2O/methanol).

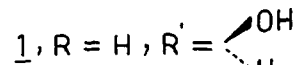
Nodososide (*1*), $[\alpha]_D$ = -21.3°, did not crystallize, and has molecular formula $C_{38}H_{66}O_{14}$, determined by combustion analysis and FD mass spectral analysis, which showed a peak at m/e 769 (M^+ + Na). On acid methanolysis it gave methyl arabinoside (g.l.c.) and a second methyl glycoside, while the aglycone was degraded to intractable material. Benzoylation with p-bromobenzoyl chloride and pyridine of the reaction mixture followed by TLC-SiO_2 separation in 20% Et_2O/hexane gave methyl 2-O-methyl-3,4-di-O-(p-bromobenzoyl)-β-D-xylopyranoside, ^{1}H NMR ($CDCl_3$, 270 MHz): δ 7.88-7.48 (m; 8; aromatic-H's), 5.51 (dd; 1; J = 8.7, 8.7; 3-H), 5.19 (ddd; 1; J = 8.7, 8.7, 4.4; 4-H), 4.40 (d; 1; J = 6.1; 1-H), 4.25 (dd, 1, J = 11.5 and 4.4, 5-H eq), 3.55 (s; 3; OMe), 3.47 (s; 3; OMe), 3.45 (br t;1H; J = 11.5; 5-H ax), 3.30 (dd; 1; J = 8.7 and 6.1; H-2), CD: 236/253, Δε + 12/ - 38, A = -50, and methyl 2,3,4-tri-O-(p-bromobenzoyl)-α-L-arabinopyranoside, ^{1}H NMR ($CDCl_3$, 270 MHz):

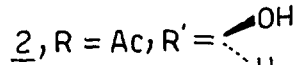
†This contribution is part of the Progetto Finalizzato "Oceanografia e Fondi Marini",CNR, Roma.

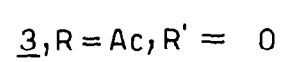
δ 7.93 - 7.42 (m; 12; aromatic-H's), 5.66 - 5.59 (m; 2; 2-H, 4-H), 5.52 (dd; 1; J = 7.3, 3.2; 3-H), 4.62 (d; 1; J = 6.0; 1-H), 4.28 (dd; 1; J = 11.5, 3.0; 5-H ax), 3.88 (dd; 1; J = 11.5, 1.5; 5-H eq), 3.53 (s; 3; OMe); CD: 236/253, Δε - 30/ + 95, A = + 125. The signs of exciton-split CD curves accompanying the two structures established the D-configuration of the xyloside and the L-configuration of the arabinoside[4]. We have assigned every sugar signal in the 500 MHz high-resolution ^{1}H NMR spectrum (D_2O) of *1* using spin-decoupling techniques and established the configuration of the glycoside linkages and that arabinose is in its furanose form: 2-O-Me-β-D-xylopyranosyl residue, 1-H δ 4.537 (d, J = 7.75), 2-H δ 3.036 (dd, J = 7.75, 9.02), 3-H δ 3.472 (dd, J = 9.02, 9.02), 4-H δ 3.631 (ddd, J = 10.3, 9.02, 5.65), 5S-H δ 3.261 (dd, J = 11.60, 10.3), 5R-H δ 3.903 (dd, J = 11.60, 5.65), OMe δ 3.605 (s); α-L-arabinofuranosyl residue: 1-H δ 5.146 (s), 2-H δ 4.153 (d, J = 3.80), 3-H δ 4.129 (dd, J = 7.20, 3.80), 4-H δ 4.034 (ddd, J = 7.20, 4.60, 3.52), 5-H_2 δ 3.761 (dd, J = 12.50, 4.60) - 3.835 (dd, J = 12.50, 3.52). Treatment with acetic anhydride and pyridine at room temperature produced an hexaacetate(*2*, 6 $\underline{CH_3}$ -C = O at δ 2.010, 2.026, 2.030, 2.083, 2.090 and 2.10) showing in the ^{1}H NMR spectrum the 2-O-Me-xyl H-2 and the arab H-2 signals essentially unshifted, δ 3.132 (dd, J = 8.75 and 7.40 Hz) and 4.180 (d, J = 3.70 Hz). These data established both the sequence and the interglycosidic linkage of the disaccharide moiety as shown in *1* . Analysis of the ^{13}C NMR spectrum provided corroborative evidence; 2-O-Me-β-D-xylopyranosyl residue: 105.2 (C-1), 84.1 (C-2), 77.8 (C-3), 71.0 (C-4), 67.1 (C-5) and 60.7 (OMe); α-L-arabinofuranosyl residue: 107.6 (C-1), 93.1 (C-2), 77.6 (C-3), 85.0 (C-4), 62.4 (C-5). Assignments have been made by comparing the spectrum of *1* with those of methyl β-D-xylopyranoside[5] and methyl-α-L-arabinofuranoside[6].

The glycosyl residue accounts for $C_{11}H_{19}O_8$ out of $C_{38}H_{66}O_{14}$ molecular formula, leaving $C_{27}H_{47}O_6$ for the aglycone moiety. ^{13}C NMR showed absence of carbon-carbon double bonds. A saturated sterol with six hydroxyl groups (four secondary and two tertiary; off-resonance ^{13}C NMR) was thus a plausible candidate for a structure assignment. In agreement with a cholestane structure the ^{1}H NMR showed methyl doublets at δ 0.907 (6H; J = 6.50; 26 and 27-H) and 0.930 (3H; J = 6.75; 21-H) and two methyl singlets at δ 0.977 (18-H) and 1.325 (19-H). A multiplet centered around 4.11 ppm had the complexity normally seen for 3β-hydroxyl group, and its downfield position, *ca.* 0.5 ppm









shifted relative to 5α-cholestan-3β-ol, along with the dd (J = 3.1, 2.5) at 3.678 ppm, characteristic of an equatorial proton coupled with two other protons, led to postulate a 3β,5α,6β-trihydroxy moiety, which is a common element in marine polyhydroxysterols[7]. Significant shifts were noted for both the angular methyl resonances of *1* when the spectrum was measured in pyridine (δ 1.849 and 1.310; cf. 1.325 and 0.977 in D_2O), indicating that both the angular methyl groups were subjected to 1,3-diaxial interaction with hydroxyl groups. This suggested location of the second tertiary hydroxyl at C-8, which is a common feature in polyhydroxysterols isolated from the same starfish[8]. The carbon chemical shifts for 5α-cholestane-3β,5,6β-triol have been published[9]. Taking this as starting structure the ^{13}C NMR shifts for carbons in rings A and B as well as for carbons 11,12 in *1* (Table) well corresponded to those expected upon introduction of an axial hydroxyl at C-8[10,11]. C-7 and C-9 (β-carbons) are downfield shifted ppm 6.0 and 2.7, respectively, C-11 (γ-carbon) is upfield shifted ppm 2.6, while C-19 (δ-carbons) which is subjected to the 1,3-diaxial $OH-CH_3$ interaction[10], is downfield shifted ppm 0.9. The chemical shift of carbon-6 (γ-carbon), downfield relative to the model compound, is consistent with the large deviation (7-10 ppm downfield) from additivity at the hydroxyl-bearing carbons found in 1,3-syn-diaxial dihydroxysteroids[11]. The third secondary hydroxyl group was located at C-15α on the basis of the characteristics (ddd; J = 12.7, 9.8, 3.0) of the hydroxymethine signal in the ^1H NMR at δ 4.324 and confirmed by the ^{13}C NMR frequencies assigned to C-14, C-15, C-16 and C-17 (Table), when compared with those of the corresponding carbons of 5α-cholestan-15α-ol[10].

TABLE - ^{13}C NMR shifts of the aglycone carbons in *1* and *2* (TMS = 0)

Compound	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1($py-d_5$)	34.3	31.8	67.3	42.4	75.7	77.9	41.8	76.7	48.7	39.1	19.4	42.4	44.8	66.3
2($CDCl_3$)	33.1	29.9	71.3	36.9	75.7	78.0	41.1	76.0	47.3	38.2	18.2	39.0	44.0	61.9

	15	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
1($py-d_5$)	69.2	40.9	55.0	15.6	18.2	35.4	18.9	31.9	27.8	83.5	30.6	18.2	18.2
2($CDCl_3$)	72.4	37.9	54.9	14.9	17.9	35.0	17.7	31.5	27.4	83.0	30.1	17.4	18.3

The remaining secondary hydroxyl group (bm at δ 3.350) should be placed in the side chain, possibly at C-24, and should be the site of glycosidation. The carbon chemical shifts for 24R- and 24S-hydroxycholesterols have been recently published[12]. Using one of these (the chemical shifts of the side chain carbons in the two C-24 epimers were only slightly different) as model compound and the glycosidation shifts reported by Tori *et al.*[13] for *sec*-alcohols the chemical shifts of the side chain carbons of *1* well corresponded to those expected. Calculations with the hydroxyl group in *22*[14] or *23*[15] positions gave values that were far off from experimental results.

The proposed formulation 5α-cholestane-3β,5,6β,8,15α,24-hexol 24-O-glycosidated for the new compound received additional confirmation by the following data.

a.- The hexaacetate *2* showed two aglycone protons α to acetoxy groups, 3α-H δ 5.20 and 15β-H δ 5.14, in the ^1NMR.

b.- Oxidation with pyridinium dichromate in CH_2Cl_2 of *2* produced a monoketone *3*, whose ^1H NMR was devoid of the 6α-H signal and showed the 19-H signal at upfield position, δ 1.00, relative

to *2*, δ 1.337, thus giving evidence for the removal of a 1,3-diaxial methyl-hydroxyl interaction in the conversion *2* → *3*, consistent with a 6β-OH assignment in *2* (and *1*).

c.- The hexaacetate *2* formed a phenylboronate; since the ketone *3* did not react with phenylboronic anhydride, the formation of the boronate ester, which involves the 6β-OH, requires one *tert*-hydroxyl be situated at the 8β-position.

ACKNOWLEDGEMENTS

We are grateful to Professor K. Nakanishi (Columbia University, New York) for FD-mass spectral analyses and to Brüker Spectrospin (Karlsruhe, West Germany) for 500 MHz NMR analyses. We also thank Mr. P. Laboute and Mr. M. Marly of the Centre ORSTOM de Noumèa and Mrs. M. Pusset of the Laboratoire des Plantes Mèdicinales du C.N.R.S. de Noumèa for the technical collaboration.

REFERENCES

1.- Part 7; R. Riccio, A. Dini, L. Minale, C. Pizza, F. Zollo and T. Sevenet, *Experientia 38*, 68 (1982).

2.- Y. Hashimoto, "Marine Toxins and Other Bioactive Marine Metabolites", pp.280-288, Japan Scientific Societies Press (1979).

3.- F. De Simone, A. Dini, E. Finamore, L. Minale, C. Pizza and R. Riccio, *J. C. S. Perkin I*, 1855 (1981); R. Riccio, F. De Simone, A. Dini, L. Minale, C. Pizza, F. Senatore and F. Zollo, *Tetrahedron Lett.*, 1557 (1981).

4.- H.W.Liu and K. Nakanishi, *J. Amer. Chem. Soc. 103*, 559 (1981).

5.- P.A.J.Gorin and M.Mazurek, *Can. J. Chem. 53*, 1242 (1975).

6.- R.G.S.Ritchie, N.Gyr, B.Korsh, H.J.Koch and A.S.Perlin, *Can. J. Chem. 53*, 1424 (1975).

7.- F.J.Schmitz, "Marine Natural Products", P.J.Scheuer ed., vol.I, chap.5, Academic Press, New York (1978); U.Sjostrand, L.Bohlin, L.Fisher, M.Colin and C.Djerassi, *Steroids 38*, 347 (1981).

8.- R.Riccio, L.Minale, S.Pagonis, C.Pizza, F.Zollo and J.Pusset, *Tetrahedron* (in press).

9.- J.W.Blunt and J.B.Stothers, *Org. Magn. Res. 9*, 439 (1977).

10.- H.Eggert, L.L.Van Antwerp, N.S.Bhacca and C.Djerassi, *J. Org. Chem. 41*, 71 (1976).

11.- C.L.Van Antwerp, H.Eggert, G.D.Meakins, J.O.Miners, and C.Djerassi, *J. Org. Chem. 42*, 789 (1977).

12.- N.Koizumi, Y.Fujimoto, T.Takeshita and N.Ikekawa, *Chem. Pharm. Bull. 27*, 38 (1979).

13.- K.Tori, S.Seo, Y.Yoshimura, H.Arita and Y.Tomita, *Tetrahedron Lett.*, 179 (1977).

14.- Y.Letorneux, Q.Khuang-Huu, M.Gut and G.Lukacs, *J. Org. Chem. 40*, 1674 (1975).

15.- The chemical shifts for the side chain carbons of 23-hydroxycholesterol have been calculated by using the cholestane side chain resonances and the hydroxyl substituent parameters given by F.W.Wehrli and T.Wirthlin in "Interpretation of Carbon-13 NMR Spectra", p.45, Heyden, London (1976).

(Received in UK 11 May 1982)